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OBSERVATIONS ON CORTICAL SOMATIC SENSORY MECHANISMS OF CAT AND MONKEY*

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INTRODUCTION

THE ELECTRICAL RESPONSES of the somatic sensory cortex set up by peripheral stimulation provide one means of studying the phenomena of the local sign. Definite results indicating the applicability of this method to the problem have been reported previously (Gerard, Marshall, and Saul, 1933, 1936, Marshall, Woolsey, and Bard, 1937, Bard, 1938). This paper will deal mainly with certain aspects of the spatial distribution of the cortical reactions set up by stimulation of a single peripheral point and recorded from the pial surfaces of the cat and monkey (*Macaca mulatta*). We place much emphasis on the fact that physiological stimuli in the form of small discrete displacements of hairs or skin were used. Electrical stimulation of peripheral nerves, which cannot be expected to give information of comparable significance for the solution of the problem, was employed only to obtain some complementary data. In addition to an examination of the responses obtained under anesthesia some observations were made on the cortical reactions of unanesthetized animals.

METHODS

Potentials were amplified by a four stage resistance capacity coupled amplifier †. The coupling condensers usually used had a capacity of 0.5 microfarad giving an overall time constant of approximately 93 msec (see following paper Fig 1, A, page 28). 4.5 microfarad condensers were available to give a time constant of approximately 0.5 sec. In the later experiments after consultation with Dr S A Talbot, common mode degeneration having a ratio of 1000 to 1 was introduced into the amplifier. Stimulus occurrence was controlled by a three channel timing circuit triggered by a frequency monitor which also triggered the x axis unit.

Tactile stimulation was applied to hair covered areas by a small camel's hair brush, to bare regions by the tip of a short section (about 1-1.5 cm) of a cat's vibrissa. These objects were mounted on a lever rigidly attached to the moving armature of an electromagnetic device the coils of which were energized by a pulse 3-5 msec in duration. This produced a regular quick to and fro movement which at the end of the lever, amounted to a displacement of approximately 0.5 mm within a few milliseconds. Arrangements were such that the movement of the stimulator occurred at a given and adjustable point on

* Certain aspects of these observations were previously reported by one of us (Bard, 1938).

† Fellow of National Research Council 1936-1938, during which time most of these experiments were done.

‡ This amplifier was quite similar to those designed by Mr Albert Grass for Dr Hallowell Davis at that time (1936) and was constructed by one of the authors while he was a guest in the laboratory of Dr Davis. Grateful acknowledgment is made for the helpful advice of Mr Grass and Dr Davis.

the x-axis line of the cathode ray tube. Thus a clear signalling of the exact time of activation of the stimulator was assured. The stimulus was repeated at regular intervals of 1 to 2 seconds. Increasing the interval to several seconds had little if any effect on the amplitude of the response. It was found that the application of the stimulator to the skin of a human subject gave a sensation of very light touch which was just above the threshold.

After opening the skull and dura so as to expose the greater portion of the external surfaces of one or both cerebral hemispheres the head of the animal was placed in a Horsley-Clarke instrument constructed to carry two electrodes. This arrangement permitted rapid and precise placing of the leads on the pial surface. In the monkey experiments the cortex was explored by moving the pick-up electrode in steps of one millimeter each along successive antero-posterior and transverse coordinates of the Horsley-Clarke instrument. The positions of the points were also recorded on an enlarged photograph of the brain. Because of the convex contours of the hemisphere the latter is the more accurate way of determining the exact positions and spatial arrangements of the points, but the plotting of an area in terms of coordinates in a horizontal plane is a sufficiently accurate and much more convenient way of arranging the data. In Fig. 2, for instance, the records do not exactly occupy the positions of the points on the brain. The more lateral points are a little more than 1 mm. apart because the mm. readings were taken on a horizontal plane.

In some of the monkey experiments, exploration of one or the other bank of the central sulcus was carried out after the opposing gyrus (precentral or postcentral) had been carefully dissected away. All exposed cortex except the portion under immediate observation was protected from drying by pieces of cellophane.

Nembutal (Abbott's "veterinary") was usually employed for anesthesia. During most of these experiments 0.3 cc. or 0.4 cc. of nembutal was administered whenever the spontaneous activity of the cortex interfered too much with the observation of the correlated response. Some experiments were done on cats and monkeys in which dial, or chlorolosan or ether was used. For each point a picture was taken while observing the cathode ray trace to ascertain whether or not the picture obtained was typical. If a spontaneous wave interfered to any marked extent the record was taken again. Observations under light or no anesthesia were made with the use of an electrode system which could be mounted in trephine holes in the skull. This system consisted of 5 silver wires embedded in bakelite cement* within a short cylinder of stainless steel. The cylinder was threaded at one end so that it could be screwed in trephine holes in a manner similar to that employed by Clark and Ward (1937). The inside diameter of the cylinder was 11.5 mm. The end brought in contact with the cortex presented a smooth surface flush with the ends of the silver wires and these were distributed symmetrically, one in the center and the others equidistant from it on the four quadrant radii. Flexible wires connected each electrode to a switch box and thus any one of the five could be switched into the circuit. Potentials were always measured against the wall of the cylinder which was grounded. The employment of dissimilar metals here is open to some objection. This factor was, however, constant and therefore may be, perhaps, disregarded. Under ether anesthesia two such units were mounted in the skull of the monkey, one on the face or hand area, the other on the trunk or leg area. The skin was loosely sutured around the electrode cylinders and the animal was then secured in a chair in the sitting position. Observations were made on the responses to mechanical and electrical stimuli. The latter were applied through an active electrode of moist cotton twine tied around a toe or digit; the indifferent electrode was attached to the arm or the leg and consisted of a large plate with a saline-soaked gauze pad under it. The electrical stimuli were used to secure more definite information on the absolutely and relatively unresponsive† periods of the reaction than can be obtained with mechanical stimuli. Data on localizations and recovery times were secured, first under ether, next after release of the ether and then for a period of one to two hours under nembutal. During the conscious period the animal appeared to be comfortable and partook liberally of food and drink from the hands of the experimenters.

* Bakelite cement is probably not suitable for use in chronic preparations, but it appeared to be satisfactory for periods up to 12 hours. These experiments were not conducted over longer periods of time.

† "Unresponsive" is used instead of "refractory" to avoid confusing the phenomena discussed here with the more rigorously defined refractory states of peripheral nerve fibers.

The pick-up electrodes used in experiments on anesthetized animals consisted of moist cotton threads drawn through steel tubing and kept wet with Ringer's solution. A similar arrangement in contact with a silent area or a connection with the Horsley-Clarke instrument served as the indifferent electrode.

RESULTS: CAT

We have found two general types of cortical response. One is the primary response with which these experiments are chiefly concerned. This response, which occurs after a shorter latency than the second type, is definitely localized, predominantly surface positive, often very nearly monophasic, and it repeats very well with each successive stimulus. It is usually the only significant response evoked by weak stimuli when nembutal anesthesia sufficiently deep to reduce considerably or even abolish spontaneous cortical activity is used. The second type of response was investigated briefly in preliminary experiments. There appear to be two kinds of secondary responses. The chief characteristic of the first of these is variability—in shape, phase, latency, and even in incidence of occurrence. Unlike the primary response, it is not always evoked by each successive stimulus and the first phase is not always positive. Detailed observations of it were made in only two cats anesthetized with chlorolosan. The latency appears to be a function of the distance from a region of maximal response for the primary wave. For example, as the electrode was moved caudally along the marginal or suprasylvian gyrus, the latency of the secondary response increased to 3 or 5 times that of the primary response of region 2 for the forefoot (see Fig. 1). The amplitude, incidence, and irregularity of all features of this secondary response increase as the anesthesia becomes lighter.

A secondary response which may be different from that just described sometimes occurs even in deep anesthesia when a peripheral nerve is stimulated by electric shocks. It has not been observed very frequently by us but we are inclined to believe that it resembles the secondary response of Forbes and Morison (1939).

Tactile stimulation of a small area on the dorsal surface of the forefoot consistently evoked at three regions on the surface of the pia of the contralateral cortex (Fig. 1, A and B) potential waves which we believe to be primary responses. Each region showed a maximal response which was usually limited to an area or spot having dimensions less than a millimeter. Occasionally potentials of a comparable order of magnitude were observed over a larger area. For instance, in the animal whose brain is shown in Fig. 1A, there were at region 1 two adjacent spots separated by a short strip from which smaller potentials were recorded. Each region characteristically consists of a maximal spot surrounded by a submaximal margin of variable extent, usually 1 to 2 mm. wide, but not necessarily symmetrical with respect to the maximal point. The two parts of Fig. 1 fairly illustrate the degree of variability found in different preparations.

The potential wave at region 1 was usually the smallest and the most variable; at region 2 the potential was usually larger and more constant; and

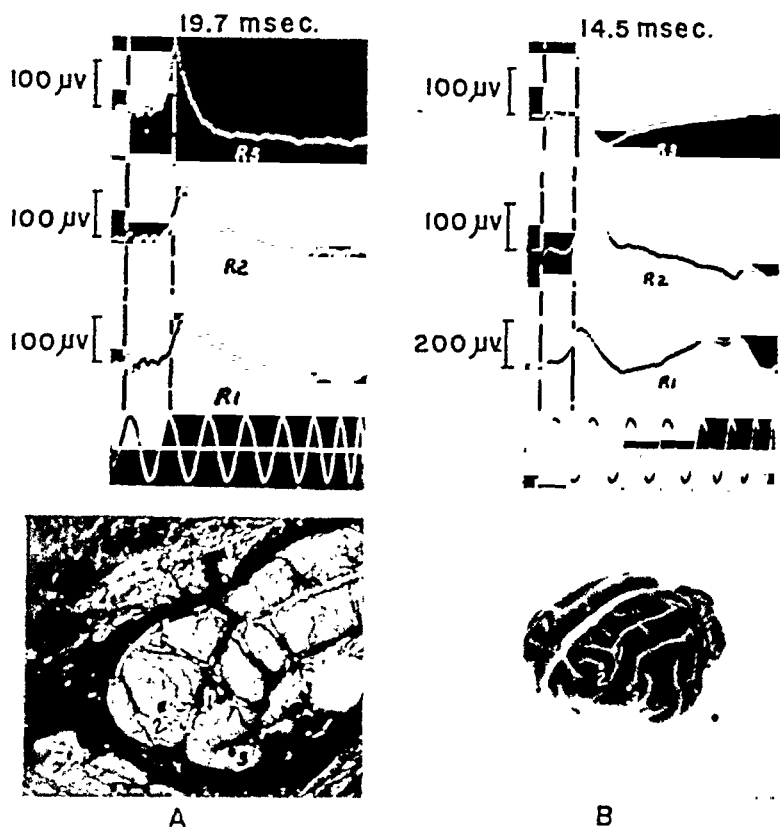


FIG. 1A. Cat, nembutal, 2 19-38. Photograph of rostral pole of left cortex of cat's brain showing regions 1, 2, and 3, and the potential records from each in column above. Stimulus used was camel's hair brush on contralateral forefoot, stimulus sequence in 2 second cycles. In this figure and all subsequent ones electrode positivity is indicated by upward deflection. Vertical lines drawn to show latency differences, and time intervals between them indicated in msec. In these and all subsequent records the mechanical lag of the stimulator is approximately equal to the duration of the signal artifact, so true latencies should be estimated from the end of the stimulus artifact. The time line represents 60 cycles per sec.

B. Cat, nembutal, 3 10-38. Same type of experiment as A. Cortical points marked on a representative picture (brain used in this experiment was not photographed). This example more typical than A in regard to differences of spontaneous activity between region 1 and regions 2 and 3.

at region 3 it was most constant throughout an experiment and showed the shortest latency, highest amplitude and most reproducible wave form. The amplitude of the responses at the three regions varied independently during the course of an experiment. As a rule spontaneous activity was much less at region 3 than at region 1 or 2 (see Fig. 1B). The difference in spontaneous activity varied in different preparations and in different stages of narcosis, but it was usually very definite. Even in quite deep narcosis, the postcruciate

area in which region 2 is located showed stray variations of small amplitude as well as groups of spike-like waves of high amplitude.

Since the tactile stimulator operated with a faint click and because of the proximity of auditory cortex to region 3, it was always necessary in studying the response here to make sure that its source was tactile and not auditory. This was done by the simple process of moving the stimulator away. If the response disappeared it was regarded as somatic; if it persisted and showed increased latency as the stimulator was moved away from the ear, it was assumed to be auditory. In some experiments the tactile response was obtained more caudally on the ectosylvian gyrus than is shown in Fig. 1 and in some cats the region responding to auditory stimuli extends well forward in this gyrus.

In several experiments the question of a primary response to tactile stimulation of the ipsilateral forefoot was investigated. Such a response was found so rarely that it seems reasonable to attribute its occurrence to some experimental error or artifact, especially in view of the great sensitivity of the contralateral reactions which makes it possible for the application of a slight mechanical stimulus to one side to set up a reaction by stimulating receptors of the other side.

The great sensitivity of these reactions in both cat and monkey demanded considerable care to prevent a slight mechanical stimulus from occurring at other than the desired region in the periphery. For stimulating fixed points in the periphery the tactile stimulating unit was clamped on a heavy stand and the latter was placed on a piece of sponge rubber. The electric cable which connected the stimulating unit to the impulse generator could not be permitted to lie on the table near the animal's body because the barely perceptible vibration imparted to the cable from the movement of the stimulator was transmitted through the table and sometimes stimulated receptors which were in contact with the table. In view of these facts the difficulty of applying proprioceptive or deep pressure stimulation is obvious. There are relatively few muscles that can be completely isolated mechanically so that a stretch stimulus can be applied which will affect that muscle only. If one attempts to excite deep pressure end organs by placing the wooden lever of the stimulator in contact with some part of the body, the tactile and proprioceptive receptors in a large part of the animal will receive a suprathreshold stimulus. Some of the sources of such artifacts have been revealed only by the masking phenomena (see below). For instance, a weak, and more or less continuous current of air will not usually produce a stimulus sufficiently discontinuous in time to evoke definite surface positive responses of high amplitude, but it may mask the response to the controlled tactile stimulus. Similarly sounds, even those of ordinary conversation, have been observed to mask the response partially or completely. Under certain conditions, opening the camera shutter a fraction of a second before the controlled stimulus occurs produces a tactile stimulus which, depending on the exact time the "click" of the shutter occurs, either sums with the controlled stimulus or partially masks it. This type of artifact could usually be controlled if the camera operator acted as a screen by standing between the camera and the animal. Irregularities in the responses due to respiratory movements were not controlled. For this reason peripheral areas which moved with respiration were not used for securing data discussed in this paper.

It is obvious that in many experiments the actual maximal foci for regions 1 and 3 may have been on the banks of the sulci. Regions lying more than 2 or 3 mm. posterior to the junction of the ansate and lateral sulci were not explored systematically. While no serious attempt was made to map the cat's cortex, as we have done in the case of the monkey, we did devote a few

experiments to an investigation of the general topography of the cat's somatic sensory cortex. The posterior part of the trunk and the hind leg are represented on the medial surface of the hemisphere and on the adjacent portion of the dorsal aspect of the cortex in the region caudal to the cruciate sulcus. The more lateral parts of the post-cruciate region and the regions around the coronal sulcus including the anterior suprasylvian and anterior ectosylvian gyri are concerned with the forelimb, shoulder, neck and face.

The area of cortex from which electrical responses can be recorded when a branch of the superficial radial nerve supplying one or more digits was stimulated by a single induction shock extended over a large part of the frontal pole. The responses to electrical stimuli also showed higher amplitude and shorter latency, than those following physiological (tactile) stimulation.

MONKEYS

In one series of experiments the entire Rolandic area of the monkey's brain was mapped by exploring with the tactile stimulator the entire body surface each time the active electrode was placed on a cortical point or spot. It was found that a given cortical spot within the sensory area thus determined may yield potentials of different sizes when a discrete tactile stimulus is applied successively to different points over a restricted peripheral zone on the contralateral side. By determining the relative intensities of these potentials we were able not only to delimit the representation of a part on the cortex, but also to make out with some accuracy the sequence of the representations of different parts and the transitions from one zone to another. The results obtained by this method, together with maps illustrating the topography of the representation of tactile sensibility, have been partially reported elsewhere (Bard, 1938a and b), and will be presented in complete form later. The experiments reported in the present paper had their origin in the observation that tactile stimulation of almost any peripheral point elicits responses over a cortical area of several square millimeters. They deal mainly with measurements of the group or groups of potentials which appear when the stimulation is at a fixed peripheral locus. While the former method is the only practical way of obtaining the necessary information to map the representation of the periphery on the cortex, the latter method gives more accurate information about the manner in which the somatic sensory cortex is activated by stimulation of any one peripheral region.

The points of maximal primary responses

On the basis of anatomical evidence, major reactions should be found in areas 3, 1 and 2 of Brodmann. According to Walker (1938) these are the regions to which the greater part of the radiations from the nucleus ventralis posterior and the nucleus lateralis posterior project. Certain physiological evidence (Bard, 1938a) led us to look for sensory reactions in area 4. We have also investigated areas 5 and 7. The question of ipsilateral representation received considerable attention. Eight experiments on monkeys were devoted principally to plotting out the total cortical area giving electrical responses

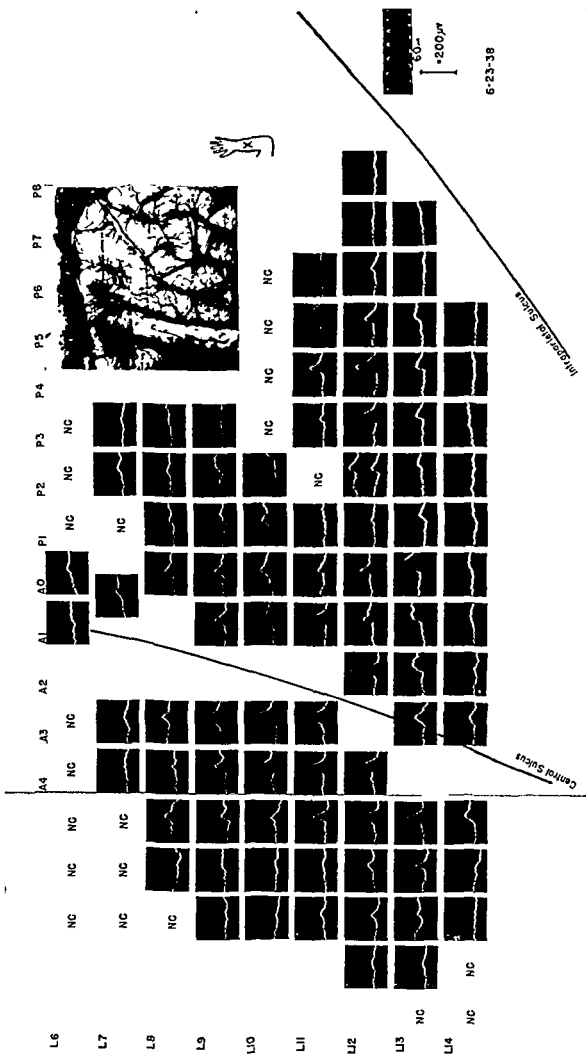
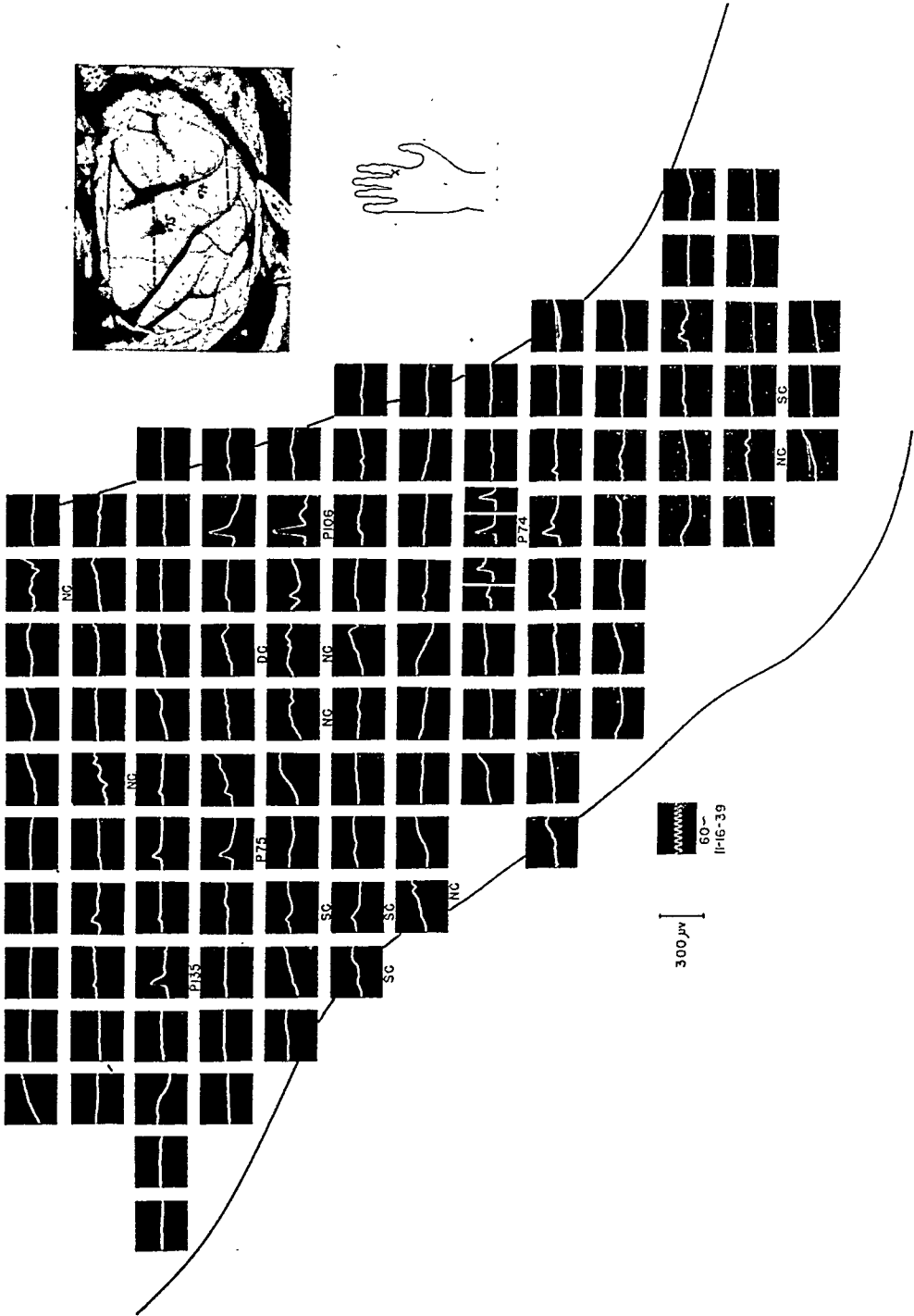


FIG. 2. Monkey, nembutal, 6/23/38. Responses on pial surface of left cortex obtained in response to equal tactile stimuli applied to dorsal surface of right forearm at point X in diagram. Area of cortex surveyed indicated between inked lines on photograph of the brain. Motor cortex removed exposing caudal bank of central sulcus (area 3). Each oscillograph record represents response at a point 1 mm. distant from all adjacent points, as indicated by the mm. coordinates on x (antero-posterior) and y (transverse) axes. Records right of central sulcus line are from dorsal surface of postcentral gyrus. Records at left of central sulcus line taken from rostral bank of postcentral gyrus and, reading to the left, indicate responses at points 1 mm. apart on the bank. Maximal potentials on bank and near rim of central sulcus have latencies of 15 to 16 msec., those at more caudal points show latencies of about 18 msec. NC = no wave correlated with stimulus.



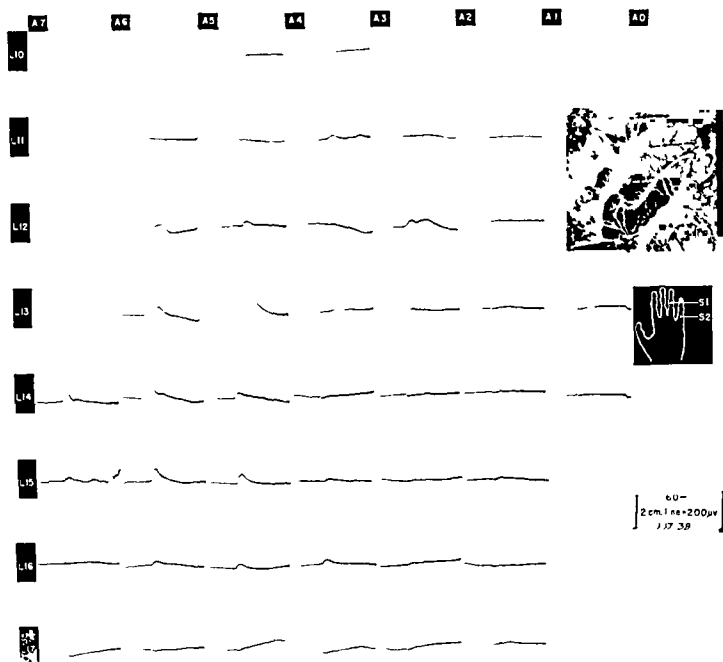


FIG 4 Monkey, nembital, 11/17/38. Distribution of action potential responses over area on left postcentral gyrus indicated on photograph of brain. The motor cortex, area 4, had been removed by subpial dissection. Pairs of tactile stimuli were delivered by two camel's hair brush units. First stimulus (S1) at point indicated on digit of right hand and followed after 17.5 msec by a second stimulus (S2) delivered at the point indicated on digit 5. The stimulus artifacts are not clear on the assembled records. The second stimulus occurred at the start of the wave correlated with the first stimulus. The response to S2 falls within the unresponsive phase of that to S1. At point A6-L13 and at other points, the D4 reaction does not completely abolish the D5 reaction. At A5-L13 the D4 response is near zero and the D5 response is nearly full-sized. The latencies of the two reactions were about the same, 14 msec for D5 and 13.5 msec for D4. Each oscillogram was taken at a point 1 mm from each of the adjacent points.

A6-L12, A6-L13, A6-L14, A5-L13, and A3-L12. The maximal point for both reactions when evoked separately was approximately at A6-L13 and at that point, as well as at most of the others where major responses to either stimulus occurred, the D4 reaction did not completely abolish the other. The exact amount of attenuation was dependent on the strength of the first stim-

ulus and did not indicate any specific separation of the neurones involved. A nearly maximal D5 reaction occurred within the absolutely unresponsive time of the D4 reaction at A5-L13, but at this point the D4 reaction was very small. Other experiments conducted in a similar manner showed the same result. It became clear that a large reaction to the second stimulus occurred only if the electrode was on a point nearly silent for the first reaction, but nearly maximal for the second. In general, this information does not appear to add anything of significant value to that obtained by plotting the distribution of reactions to a single stimulus. It was found that however the attenuating stimulus was arranged in the periphery the maximal reactions are relatively resistant to attenuation.

The experiment illustrated in Fig. 4 is, perhaps, most useful in showing that the almost total absence of the D4 reaction at point A5-L13 was not due to local depression of the cortex at that point, since the D5 reaction was nearly maximal. This result also illustrates the general fact that in areas 3 and 1 cortical spots which do not respond to any peripheral point were rarely encountered.

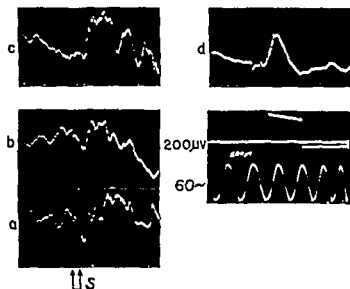
Another method of demonstrating specific overlap consists in application of the masking effect (Marshall, Woolsey, and Bard, 1937). If continuous tactile stimulation is applied at a point on the periphery near the point at which a discrete tactile stimulus is being applied the specific response to the latter becomes smaller or disappears. In this case the attenuation or abolition of the response is primarily due to equilibration effects, since neurones common to both reactions are kept more or less completely occupied by the continuous stimulation, and at any one time only a few are available for participation in the reaction which follows the discrete stimulus. The long and definite recovery times of these reactions favor the masking effect. Masking can usually be demonstrated wherever overlap occurs. It indicates convergence of central pathways since it can be obtained by stimulating such widely separated peripheral regions as thumb and face which have partially overlapping cortical representations.

The effects of anesthesia

Four experiments were done on monkeys to observe the effects of the anesthetic on the amplitude, latency, recovery time and distribution of the responses. Under ether anesthesia two groups of electrodes, as described above, were screwed into trephine holes in the skull. One electrode group was placed on the postcentral gyrus near the medial rim (foot and leg area), the other just medial to the end of the intraparietal sulcus (face and hand). The animal was then secured in a conventional monkey chair and observations were made from each of the five electrodes of each group while the periphery was explored with the tactile stimulator to find the cutaneous area which was maximally represented under each electrode. These electrodes were theoretically less sensitive to the alpha rhythm than those used in the experiments previously discussed, but their sensitivity to spontaneous activity of beta frequencies was probably about the same.

The evoked responses thus obtained under ether were similar, as regards amplitude and latency, to those obtained in the experiments under nembutal which have been discussed above. They were, however, more difficult to observe because of the much greater spontaneous activity which made precise delimitation of the submaximal margin impossible. A small response under nembutal can be seen distinctly, but the presence or absence of a small

FIG 5 Monkey, 1/27/38 No anesthesia responses evoked by tactile stimulation of face. Records *a* and *b* show two successive records of response to slight displacement of only two vibrissae. Record *c* shows response to a somewhat stronger stimulus, the camel's hair brush imparted slight movement to several vibrissae. Record *d* taken with approximately same stimulus as for record *c* 3 hours after record *c* was taken and 1 hour after intraperitoneal injection of 0.4 cc nembutal per kg. S indicates stimulus artifact.



response in the etherized (or the unanesthetized) animal is very difficult to determine. The spontaneous activity under ether could be reduced to an intensity somewhat comparable to that seen under nembutal only by pushing the anesthesia to dangerous depths. It is perhaps significant that in extremely deep ether anesthesia the responses persisted with about the same amplitude and latency.

After release of ether similar observations were made on the responses to tactile stimulation for periods up to two hours (Fig. 5). In this case also the primary responses were essentially similar to those seen under nembutal, but because of the spontaneous activity it was not so easy to determine the relationship of the wave to the stimulus. In general, the difficulty of observation due to spontaneous activity was less than under ether. The primary surface positive wave was sometimes immediately succeeded by a definite negative wave, and, 10 to 40 msec. later, a second and third diphasic wave sometimes occurred. Each wave in the total response was sharper and contained more small spike-like components than are seen under nembutal or ether. The latencies and amplitudes, however, were about the same as those observed under nembutal.

The extent of the submaximal margin in the unanesthetized animal was, as in the case of ether anesthesia, difficult to determine so we cannot definitely say whether under these conditions the cortical representation of a peripheral point is larger than under nembutal. It appeared, however, to be about the same.

Certain of the above observations as well as the effect of the anesthetics

MONKEY

Elec. stim. TOE I

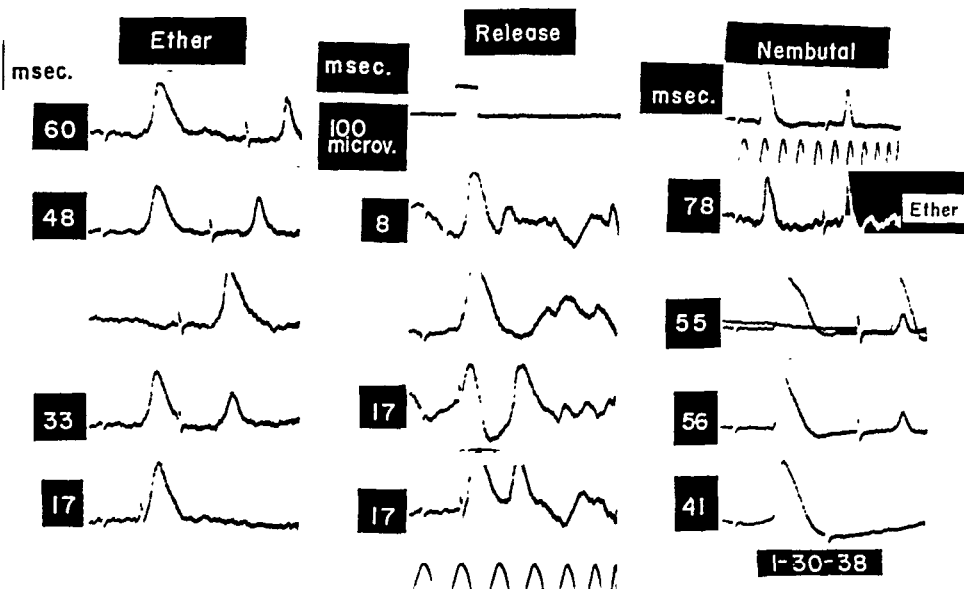


FIG. 6. Monkey, ether-release-nembutal series, 1/30/38. Trephine hole leads on post-central gyrus near central sulcus and close to medial rim of hemisphere. Electric shocks applied to toe I. Records in *column at left* indicate recovery time under deep ether. At 17 msec. second shock produces no response. The third record from the bottom represents control with second shock alone. *Middle column* shows responses obtained 4 to 6 minutes after removal of ether when animal was making spontaneous movements and displayed other signs of consciousness. At 8 msec. interval there is a definite response to second shock. The third record from the bottom represents control in which only the first stimulus was applied. In the *column at the right* the top record (interval 78 msec.) and the three lower records indicate recovery times 12–15 minutes after intraperitoneal administration of nembutal (0.4 cc. per kg.). Record at bottom of column shows interval between stimuli at which second response is near zero. Third record from bottom shows two traces superimposed: the response to a second stimulus after interval of 55 msec. is shown on one and the other represents control in which only the second shock was applied. The two uppermost records of the right column were taken with a slower trace as indicated by the 60 cycles time line between them. Top record was taken about same time as lower three and is to be compared with the ether record below it, which was taken at the same time as the group in the left hand column. Note the difference in size of the second response and the greater amount of spontaneous activity of beta frequency in the ether record.

The latencies of the first response in the ether and nembutal records are practically the same, 17 msec. The latencies after release of ether are about 16.5 msec., a difference of dubious significance. The latencies of the second responses are not significantly different from those of the first responses.

on the recovery time of the reactions can best be illustrated by Fig. 6 and 7. In this experiment, as in the others of this group, observations were made to determine at which electrode tactile stimulation of a digit or toe was maxi-

mally represented while the animal was under ether. Then stimulating electrodes were applied as described above and identical shocks applied in pairs at controllable intervals. The intensity of the shocks was set at a value which gave cortical responses of moderate amplitude with little or no overt reflex or direct muscular movements of the member. These latter complications cannot, however, be definitely excluded.

The recovery times obtained in one of the experiments with the animal first under deep ether anesthesia and later under nembutal are shown in Fig. 6. The records in the right hand column were taken under nembutal two hours after the records in the left hand column had been taken under ether —(all records in the right hand column were taken under nembutal except the one marked "ether"). It is apparent that there are only two obvious differences between the ether and nembutal records. The ether records show more spontaneous activity and a shorter absolutely unresponsive time. It is to be noted that the ether was relatively deeper than the nembutal anesthesia, for, in the monkey, the extent of elimination of spontaneous activity which can be secured by ordinary dosages of nembutal can be obtained under ether only by pushing the anesthesia to a degree not compatible with long maintenance of the preparation. Even so, the recovery time under ether is about one-third that under nembutal. The latter was administered intraperitoneally in a little less than the standard surgical dosage used in this laboratory (0.4 cc. per kg. of body weight).

The records in the middle column of Fig. 6 were taken about 5 minutes after removal of the ether mask and at a time when the animal was making spontaneous movements and showing many signs of consciousness. At this stage and occasionally at longer intervals after release from ether, the recovery times, while much shorter than during the anesthesia, are definitely measurable. In this case, a definite response was obtained when the second stimulus followed the first by 8 msec., and at 17 msec. the second response was about equal to the first. When however, the animal is more completely awake the picture of one simple surface positive response with a long and definite unresponsive time completely disappears. First, the records show more spontaneous activity and then either the refractoriness disappears or a form of summation dominates the refractoriness. The summation effects which often appear at this time are shown in the right hand column of Fig. 7. At 61 msec. they were, in this experiment, pronounced and as the stimulus interval was shortened they became greater. Negative waves and a definite multiple response had not yet appeared when these records were taken approximately 12 minutes after release of ether.

Twenty minutes or more after release of ether the animal eats and drinks from the observer's hands, sometimes vocalizes if he sees food anywhere in the room, exhibits every sign of complete consciousness and appears to be quite comfortable. The records taken under these conditions (Fig. 7, left hand column) show more spontaneous activity of both high and low frequency, and the responses to single shocks tend to be multiple. Also summa-

response (apparently similar to that described by Forbes and Morison, 1939) is more reproducible and has a longer latency, but we have only occasionally seen it. It is seen only when spontaneous cortical activity is small or absent. Its absence in our experiments is probably due to the fact that practically all our observations were made under the conditions of equilibration which exist at a cycle frequency of 1 to $\frac{1}{2}$ per second. According to the data of Forbes and Morison the recovery time of this type of secondary response might tend to prevent its appearance when the reaction is equilibrated at these frequencies.

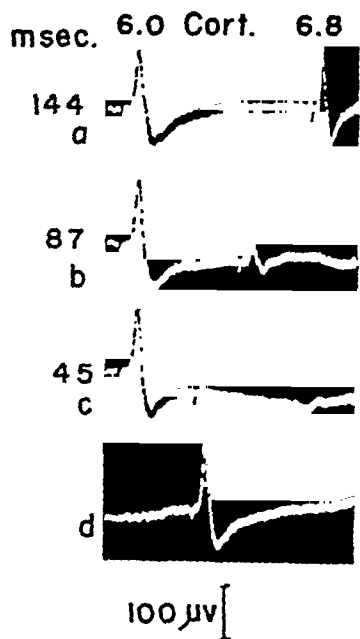


FIG. 8. Cat, nembutal 3/23/38. Same experiment as illustrated in Fig. 4 of following paper. Cortical responses at region 3 to two electric shocks applied to superficial radial nerve at intervals indicated at left of records. Stimulus cycle, 4 seconds. Latencies are indicated over 1st and 2nd responses. Note that second response is maximal at a stimulus interval of 144 msec. and nearly zero at one of 45 msec. Record *d* shows control in which second stimulus was applied alone.

Whether or not the primary response of Forbes and Morison (1939) is essentially the same as the primary response considered here has been questioned (Forbes and Morison, 1939, pp. 122-133) on the basis of the relation of frequency of stimulation to response amplitude. In a previous paper (1937) we stated that with mechanical stimulation of hairs the response disappeared, *i.e.*, was no longer visible on the oscillograph trace, at a frequency of 12 to 15 per second. Mechanical stimulation as used by us, is not a satisfactory method for the study of either recovery times or equilibration and fatigue effects, since at these frequencies the vibrating brush does not apply a temporally discrete stimulus. The values obtained with this method of stimulating are undoubtedly too low, and therefore electrical shocks to peripheral nerve trunks were principally used in the investigation of recovery times (see the following paper).

Forbes and Morison found that, at a stimulation frequency of 7 per second (electrical shocks applied to the sciatic nerve) the amplitude of their primary cortical response in the cat under nembutal was more than half the initial amplitude, and on this basis they argue that the response should not decline to extinction at double that frequency.

Forbes and Morison, however, cite values obtained under conditions of equilibration and these values do not give definite information about the absolutely and relatively unresponsive periods because the reaction merely fractionates as the stimulus frequency is increased and eventually becomes invisible on the oscillograph. We therefore wish to refer to an experiment on a cat under nembutal which

illustrates the extent of the reduction of the response to the second of a pair of stimuli as the interval is progressively decreased. In this experiment the pairs of stimuli were electrical shocks applied to the superficial radial nerve at a cycle frequency of $\frac{1}{4}$ per sec. (1 cycle in 4 sec.). The shocks were stronger than necessary to produce a maximal cortical reaction. At an interval of 144 msec. (equivalent to a frequency of 7 per sec., disregarding fatigue effects) the response was full sized (Fig. 8a). At an interval of 87 msec. (equivalent to a frequency of 11.5 per second, if we disregard equilibration) the second response was very much reduced (Fig. 8b) and at 45 msec. (equivalent to a frequency of 22 per sec., disregarding equilibration) it was barely discernible (Fig. 8c). It is clear that as the stimulus intervals are shortened to bring the second stimulus progressively further into the relative recovery time the response rapidly decreases. Equilibration at frequencies equivalent to these intervals yields amplitudes for each wave which are somewhat greater than those shown for the respective second responses, but it appears that the actual amplitude at any given frequency is that which can be predicted from simple fractionation plus possible fatigue effects. These considerations indicate that the individual responses should rapidly decrease as the stimulus frequency is increased above 7 per second.

In all of our experiments the primary foci were carefully located by restricted physiological stimulation before determinations of recovery time or other observations on responses to electrical stimulation were made. The positions of the maximal potentials evoked by electrical stimulation of the superficial radial nerve do not always exactly coincide with the positions determined by physiological stimulation, but they are always very near one another. When electrical shocks are used some potential complex can be picked up almost anywhere on the frontal pole of the cat's brain. Some part of this potential is lead artifact and part of it is still observed when differential leads are used. It is obvious that placing the active electrode on or near one of the maximal spots greatly reduces the probability of observing side chain reactions (avalanche conduction) which are recruited in significant numbers through any synaptic region when a strong stimulus is used.

It must be emphasized that when widely separated electrodes and strong stimulation are used it is possible to pick up from the pial surface a small potential which is due to activation of tracts distal (or caudal) to the thalamic synapses. This is a common finding in the optic system whether a photic or an electrical stimulus is used (Marshall, Talbot and Ades, unpublished). Possibly a part of the primary response of Forbes and Morison (1939) represents the invasion of the cerebrum by the reaction. Under the conditions of our experiments the lemniscus contribution to the reaction picked up at the cortical lead is very small or negligible. In Fig. 7c for example, the very small response to the second stimulus may be due to the lemniscus action potentials, which at that stimulus interval would be nearly full sized (see following paper). Considerations such as these lead us to believe that our primary cortical response represents invasion of the cortex by the reaction.

maximal margin of the reactions set up by stimulation of a peripheral point in the neighborhood of A. Yet the fact that the general distribution of the responses set up by stimulation of a peripheral point shows a high degree of stability is in entire accord with the idea that the pattern of response is based on a stable and anatomically specific system of neurones.

The data secured in experiments on etherized and on unanesthetized monkeys answer certain questions concerning the nature of the primary response. It is clear that a large part of the difficulty in making observations on such preparations is due simply to the coincident and unrelated occurrence of spontaneous cortical activity or "brain waves." Since the evoked response is not *always* affected by a coincident spontaneous wave or one which occurs just previous to it, it follows that the spontaneous wave which is led from that electrode, does not always occupy, or modulate the activity of, the same neurones which participate in the evoked response. But attenuation or amplification of the evoked response by a preceding spontaneous wave occurred sufficiently often to indicate that the sensory neurones involved in the cortical reactions are also involved in the elaboration of, or are modulated by, some of the brain waves. Therefore we assign part of the variability of the primary response in the unanesthetized animal to a simple interference effect and part of it to physiological interaction between spontaneous activity and the primary response.

One of the important questions concerning the primary cortical sensory reaction obtained under nembutal is whether its consistency of occurrence and amplitude are altered by the anesthetic. This question is important because, with a quiet cortex resulting from deep anesthesia, the high amplitude and low threshold of the primary reaction suggest that the excitability of the sensory neurones responsible for it is not markedly decreased by the narcotic. If we assume, as suggested above, that some of these neurones participate in the spontaneous cortical activity of the unanesthetized animal the reduction of such activity under anesthesia, especially barbiturate anesthesia, would mean that they are relatively idle and always ready to discharge when activated by the afferent impulses set up by the peripheral stimulus. If this assumption is true, the prominence and regularity of the primary reactions under barbiturate anesthesia are essentially anesthetic artifacts, and the reactions would be smaller in the unanesthetized animal or in an animal under ether in which spontaneous activity is present to a marked degree. A comparison of the results obtained in unanesthetized animals with those secured in animals under ether, nembutal, dial or chloralosan gave an unequivocal answer to this question. Regardless of the presence or absence of anesthesia (any of the anesthetics used) primary surface positive reactions of the same order of magnitude were consistently evoked by weak tactile stimuli. When light nembutal anesthesia was deepened the responses were usually reduced somewhat, but never by more than about 20 per cent. Since the method of tactile stimulation was not sufficiently quantitative over long periods of time to permit us to claim that these measurements were com-

pletely decisive, we obtained further information by stimulating a peripheral nerve with supramaximal shocks first under conditions of light nembutal anesthesia and then under nembutal sufficiently deep to considerably reduce spontaneous activity. The results were in accord with those obtained with tactile stimuli, *i.e.*, the decrease in amplitude was at most not more than 20 per cent; usually it was much less. In all these experiments it was found that the latencies of the primary cortical responses were not significantly changed by any anesthetic. The records in the three columns of Fig. 6 illustrate this point.

Another question of importance is whether the degree of localization and the spatial distribution of the primary cortical reactions to tactile stimulation of a single peripheral point are the same in the unanesthetized monkey as in the animal under nembutal. While extensive exploration of the cortex was impossible with the groups of five electrodes used in the experiments on unanesthetized animals, the maximal (or nearly maximal) primary responses which we observed in the four monkeys studied after release of ether occurred at approximately the same cortical points as did those evoked by the same stimulation in other monkeys under nembutal anesthesia. Further, the limited exploration permitted by the five electrodes of a group showed that in the absence of anesthesia, just as with nembutal, areas giving little or no response lie very close to every spot of maximal response. As already pointed out, the extent of the submaximal margin is almost impossible to determine in the unanesthetized animal because of the difficulty of observing small evoked responses against the background of large spontaneous waves (Fig. 5*a, b*). Obviously the limited number of electrodes applied has not permitted detailed determinations of the spatial distribution of the primary cortical reactions to tactile stimulation of a single point. In spite of their deficiencies in certain aspects the results obtained in the absence of anesthesia indicate that the characteristics and the distribution of the cortical responses observed under nembutal have real physiological significance.

It is clear that in the somatic sensory system the primary cortical response is a distinct entity, and that the response to cutaneous stimulation does not consist merely of an alteration in frequency and amplitude of the spontaneous activity as claimed by Kornmüller (1937) and others. It is also clearly evident from our results as well as those obtained by Gerard, Marshall and Saul (1936) that the cat possesses a definite cortical representation of cutaneous sensibility, a fact which was questioned by Kornmüller because of his failure to observe that discrete responses are evoked by stimulation of the skin.

Although the primary response is little affected by anesthesia the reactions which follow it, and presumably result from it, are clearly influenced by anesthetics. The first type of secondary response was observed and investigated in only two experiments, which were done under light chloroform anesthesia; the second type was found only under deep nembutal anesthesia. The multiple response illustrated in Fig. 7 was occasionally observed in

response to tactile and electrical stimuli in animals under nembutal, but it was never as prominent as in the absence of anesthesia. Another significant effect of anesthesia is on the recovery cycle. In the observations on monkeys which were made after release of ether and then with nembutal anesthesia, electric shocks were applied to a toe or digit, a method which is inferior to that used in securing data from anesthetized animals (Fig. 8) for the reason that the successive shocks are probably not as regularly reproducible and also because there is no way of determining their strength in relation to the maximal for the cortical response. If under nembutal submaximal shocks are applied to the superficial radial nerve, a second response may be obtained at any interval after the first stimulus, but the size of the second response decreases as the intervals are made shorter. In some experiments on unanesthetized monkeys the response to the second shock, after passing through a minimum at an interval of approximately 20 msec., increased in size as the stimulus interval was further shortened. Hence, the failure to demonstrate an absolutely unresponsive period was not due to the use of submaximal stimuli, but rather to the fact that facilitation effects intervened to increase the excitability of neurones which were in the subliminal fringe of the first reaction. Thus the apparently shorter recovery times found in light ether and in the unanesthetized animals may have been due to a shorter recovery time of all or a fraction of the neurones concerned in each successive reaction. Also the facilitation effect may bring into action neurones which in nembutal anesthesia are always unresponsive to that stimulus. Probably both of these conditions operate to produce the effect under consideration.

SUMMARY

1. The application of a brief tactile stimulus of low intensity to a very small cutaneous area evokes discrete surface positive potential waves in specific places on the contralateral cortex of the cat or monkey. These potentials constitute the primary cortical response to sensory stimulation.

2. In the cat a weak tactile stimulus applied to hairs on the dorsal surface of the forepaw (the only peripheral area given detailed examination) evokes separate maximal primary responses at three regions on the contralateral cortex. In the monkey the application of such a stimulus to any part of the body always evokes, within the cortical area of representation of that part, primary responses at two or more regions. The response having the shorter latency occurs in area 3 (Brodmann) the other with slightly greater latency is usually found in area 2. A third region of maximal primary response is often found in or very near area 1.

3. Each region or spot of maximal primary response is surrounded by a fringe or margin of submaximal responses. The overlap of the submaximal margins for peripheral regions which are adjacent or which have adjacent cortical representations is reciprocal and definite. Demonstrable interaction is usually found within the overlap.

4. Nembutal and certain other anesthetics greatly reduce the sponta-

in place and a laminectomy performed. Several spinal nerves, usually three or four in each experiment, were prepared by severing them from the cord and excising the dorsal root and ganglion. After insulation from surrounding tissues, the distal cut end of the ventral root was stimulated by accurately controlled shocks from a thyatron stimulator. These were usually maximal or supramaximal. A rate of 2 per sec. was used to avoid additive effects between successive shocks (Marraszi¹¹), and thus make the length of the period of stimulation unimportant. When this was controlled and the rate was varied up to 40 per sec. the results did not differ from those presented.

The activity resulting in the peripheral nerves of the limb in question was detected by electrodes, placed on the main branches, and connected to the amplifier (Fig. 1). The

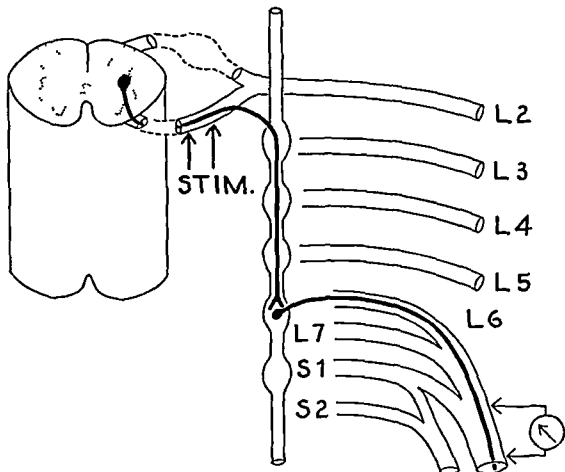


FIG. 1. Diagram showing site of stimulation of distal cut end of ventral root and of recording from sciatic nerve in the thigh.

C and B waves thus recorded enabled the mapping of the exact roots through which sympathetic fibres passed to the limbs, and by recording from the contralateral side data were obtained on the extent of extraspinal crossed pathways in such outflows. With a conduction distance of 15–35 cm. and a camera speed of 7 cm. per sec., a good separation was obtained between the B and C waves, while A waves would have been incorporated (though still discernible) into the end of the shock artifact.

Since all slowly conducting fibres are not necessarily autonomic, the possibility that some of the C and B waves might have originated elsewhere was considered and excluded by the fact that the intravenous injection of nicotine, which in the doses given (10–30 mg.) acts at autonomic ganglia without affecting postganglionic or somatic nerve trunks, was effective in blocking the previously recorded impulses. This eliminated any slowly conducting somatic efferents as a source of the potentials. It also constituted a check that any current spreading along the ventral root did not stimulate the distal cut stump of the dorsal root, and initiate an antidromic impulse in a slowly conducting afferent fibre. Existing anatomical knowledge indicates that the majority of roots stimulated do not contribute afferent or somatic efferent fibres to the limbs.

The good muscular responses obtained indicated that the animals were in good condition. When the muscle contraction interfered with the recording it was eliminated by cutting of appropriate nerves. At the termination of each experiment the roots stimulated

were checked by autopsy, at which time the total number of thoracic and of lumbar vertebrae was noted (later checked by x-ray), and a dissection made to record the exact fixation of the lumbo-sacral plexus, according to the definitions given by Sherrington¹⁴ and by Zuckerman.¹⁶

RESULTS

Cat (Lower limb). The findings in the cat have already been presented in some detail in the preliminary note and are summarized here in condensed

COMPARISON OF SYMPATHETIC PREGANGLIONIC OUTFLOW TO THE LOWER LIMB IN					
CAT		AND		MONKEY	
No. of Exp.	Positive Responses C or B waves	Root	No. of Exp.	Positive Responses C or B waves	
1	0	T 7	3	0	
1	0	T 8			
3	0	T 9			
4	0	T10			
Total Outflow	9	T11	3	1†	
	8	T12	3	2	
	7	T13*	3	3 6 5 } Major Outflow	
	6	L 1			
	9	L 2			
	4	L 3			
	3	L 4	3	0	
	2	L 5	3	0	
	2	L 6	1	0	
	1	L 7	1	0	

Fig. 2. Table of results in cat and monkey (lower limb).

* No T13 root in the monkey.

† Only 11 thoracic roots in this animal.

tabular form (Fig. 2). The results show that the preganglionic outflow to the lower limb via the sciatic nerve emerges from the spinal cord by the ventral roots of T11-L4 (inclusive) and that the roots giving the greatest contribution are T13-L3 (inclusive) of which L1 and L2 invariably give a response. T11 rarely contributes and T12 and L4 only occasionally.

From the positive roots B (10-20 m. per sec.) and C (1-2 m. per sec.) waves, or C waves alone, were recorded. The C waves appeared in all cases except two where the animal was in poor condition. In these apparently only the fibres of lower threshold responded, giving B waves alone. When the stimulus elicited both B and C waves, the B wave was much smaller in both height and area than the C wave as shown in Fig. 3 where, following each

sharp downward deflection, which is part of the stimulus artifact, appear a small B and a large C wave. These are indicated at the beginning of the record by an arrow and the letters B and C respectively. Nicotine injected intravenously in doses of 10–30 mg blocked both C and B waves from these roots. Complete abolition by nicotine of the responses from L1 is seen in Fig. 3.

Recording from the medial and lateral popliteal divisions of the sciatic nerve separately gave the same result as recording from the combined nerve. No responses from the contralateral sciatic nerve were ever obtained, if care was taken to avoid spread of current. Crossed pathways, between the sym-



FIG. 3. Effect of nicotine on sympathetic potentials of cat's sciatic nerve. Postganglionic responses on stimulating ventral root of L1 *before* (UPPER LINE) and *after* (LOWER LINE) injection of 10 mg of nicotine. \uparrow = stimulus artifact. B = B wave, C = C wave. time in $\frac{1}{2}$ sec.

pathetic chains, of fibres destined for the opposite sciatic nerve were therefore not demonstrated.

Monkey (Lower limb) The results for the monkey have been tabulated and placed alongside those of the cat for comparison (Fig. 2). Stimulation of T10 gave no response. C waves appeared only once on stimulation of T11 and this animal turned out to have 11 thoracic and 7 lumbar vertebrae, so that T11 was the last intercostal nerve. Stimulation of T12, L1, L2 and L3 gave C ($\frac{1}{4}$ –1 m per sec) responses in all experiments, the responses from T12 being smaller in amplitude than those from the lower roots. In addition small B (8 m per sec) waves, which were not nearly so conspicuous as they had been in the cat, were occasionally obtained. Stimulation of roots below L3 never yielded sympathetic potentials. The outflow of preganglionic fibres to the lower limb in the Rhesus monkey would therefore appear to be from T12–L3 (inclusive), with T11 participating under exceptional circumstances, and T12 giving a smaller contribution than the other roots. Thus whereas 7 spinal roots in the cat participate in the preganglionic outflow to the inferior extremity, only 4 roots contribute in the monkey. The outflow in the monkey

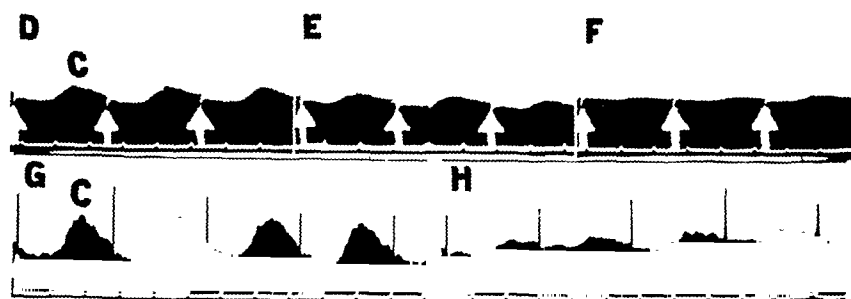


FIG. 4. Effect of nicotine on sympathetic potentials of monkey's sciatic nerve. Post-ganglionic responses on stimulating ventral roots of L3. Upper line (D E F) = low gain; Lower line (G H) = high gain D & G before E immediately after injection of 10 mg. of nicotine, and F & H 5 min. later. \uparrow = stimulus artifact (reversed in GH) C = C wave; time in $\frac{1}{2}$ sec.

is therefore more restricted. Nicotine injected intravenously in doses of 10–30 mg. blocked the responses from these roots. Figure 4 shows the effect of 10 mg. of nicotine on the C wave obtained by stimulating L3. The upper line, taken at lower gain, shows an immediate reduction in response after nicotine (E), and a more complete block five minutes later (F). The lower line shows a higher gain record of the control (G), and the degree of block five minutes after the injection of nicotine (H).

As in the cat, no responses were obtained from the contralateral sciatic nerve. There appears therefore no evidence substantiating the belief that

Ventral Root	No. of Exp.	Positive Responses (C or B) Total	Analysis of Positive Responses					
			Median Nerve		Ulnar Nerve		Radial Nerve	
			No. of Exp.	Positive Responses	No. of Exp.	Positive Responses	No. of Exp.	Positive Responses
C 8	1	0	1	0	1	0	1	0
T 1	2	0	2	0	2	0	2	0
T 2	3	0	3	0	3	0	3	0
T 3	4	0	4	0	4	0	4	0
T 4	4	2	2	0	3	2	4	2
T 5	6	Total Outflow	6	3	4	4	6	4
T 6	5		5	2	5	5	4	4
T 7	4		4	2	4	4	4	4
T 8	4	3	2	1	3	2	4	2
T 9	3	0	3	0	3	0	3	0
T10	2	0	2	0	2	0	2	0

Fig. 5. Contribution of sympathetic potentials from thoracic ventral roots to the upper extremity in the monkey

functional pathways to the lower limb cross between the sympathetic chains. Recording from the medial and lateral popliteal divisions of the sciatic nerve separately gave the same result as recording from the combined nerve, and this also agrees with the findings in the cat.

Monkey (Upper limb). The outflow of sympathetic preganglionic fibres to the upper limb was confined in the monkey to T4-T8 (inclusive), with the major outflow coming from T5, T6, and T7 (Fig. 5). The contributions from

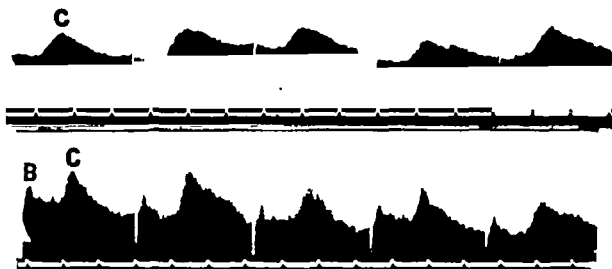


FIG. 6. Sympathetic potentials in the arm (monkey). Upper line—C waves in ulnar nerve on stimulating ventral root of T6. Lower line—B and C waves in median nerve on stimulating ventral root of T7. ↑ = stimulus artifact; B = B wave; C = C wave; time in $\frac{1}{2}$ sec.

T4 and T8 were less constant and smaller in magnitude. From above T4 and below T8 sympathetic potentials were never obtained in the nerves of the arm. There appeared to be no definite difference in the *extent* of total outflow to the median, ulnar and radial nerves. Variations in the fixation of the brachial plexus did not seem to have any bearing on the extent of outflow.

All the positive roots gave C ($\frac{1}{4}$ –1 m. per sec.) waves (Fig. 6). Occasionally a B (8–10 m. per sec.) wave was also recorded from the ulnar and median nerves. The lower line in Fig. 6 shows an unusually good example of B waves. Only once was a small B response picked up from the radial nerve. Nicotine in doses of 10–30 mg. injected intravenously blocked the sympathetic responses in the median, ulnar and radial nerves from stimulation of the positive roots.

All the monkeys, except one, proved on autopsy and x-ray examination, to possess the usual number of thoracic (12) and lumbar (7) vertebrae. In one monkey where there were only 11 thoracic vertebrae and the usual number of lumbar, the outflow to the lower extremity extended up as far as T11, the last intercostal nerve in this instance. The variations in the fixation of the lumbo-sacral and brachial plexuses were not associated with any obvious differences in the preganglionic outflows to the limbs.

DISCUSSION

Langley's work^{6,7,8,9,10} was carried out exclusively on cats under chloroform and ether anesthesia. For the lower limb he stimulated T10-L4 (inclusive) and observed the sweat formed on the pads of the feet. Although variations existed, the maximum number of sudomotor fibres emerged in L1 and L2, some in T13 and still fewer in T12 and L3. In a later paper (1894) he added a contribution from L4 in animals with a "posterior" type of (post fixed) lumbo-sacral plexus, but there appeared no constant relation between the arrangement of the plexus, and the position of the uppermost nerve containing secretory fibres for the lower limb. In a study of the vasomotor nerves to the limbs Langley reported that the maximum pallor occurred in the hind foot from stimulation of T13, L1 and L2, and in his later paper (1894) he extended the outflow of vasomotor fibres to the inferior extremity to T12-L4; only once did he note any vascular change on stimulation of T11. In the fore-foot of the cat he obtained profuse sweating by stimulation of T6, T7, T8 and slight sweating on stimulation of T4, T5 and T9. The largest sudomotor outflow occurred in T7. Vasomotor effects were obtained from the same roots, the sudomotor and vasomotor outflows coinciding very exactly. In the fore-foot then no vascular or secretory effects were ever observed by Langley following stimulation of roots above T4.

Bayliss and Bradford² adopted the plethysmographic method for recording vascular changes in the limb. Dogs under chloroform anesthesia and curare, and with the plethysmographic cuff reaching above the middle of the thigh, showed vasoconstriction in the lower limb from stimulation of the spinal roots from T11-L3 inclusive. It was least effective from T11 and L3. Stimulation of T10 and L4 had no effect. With the exception of the absence of any contribution from L4 their results corresponded closely with those of Langley in the cat. In the fore limb of the dog with the plethysmograph extending as high as the axillary folds, Bayliss and Bradford recorded a reduction in volume of the limb following stimulation of T4-T11 (inclusive), and found the maximum result occurring after stimulation of T6, T7 and T8. Stimulation of T2 had no effect. Stimulation of T3 only occasionally produced a slight diminution in volume of the limb. Stimulation of T11 gave slight diminution in volume of the upper limb only after the splanchnic nerves had been cut, which eliminated the predominant pressor effect from vasoconstriction in the visceral bed.

Our results in cats agree closely with these analyses of Langley, and Bayliss and Bradford based on the outflow of sudomotor or vasomotor fibres to the lower limb. In the Rhesus monkey the outflow appears to be restricted to fewer spinal nerves. This may perhaps be related to the assertion that the cat is more sympatheticotonic than the monkey, although it is quite possible the results do not indicate a smaller outflow but only a condensation.

Derom³ (1938), working in Heyman's laboratory, has utilized the carotid sinus reflex for recording vasomotor changes in the lower extremity. In dogs

under chloralose anesthesia, the pressures in the femoral artery and vein, and carotid artery were recorded manometrically. Section of the rami communicantes of the upper three lumbar nerves abolished the vasomotor effects in the corresponding lower limb ordinarily initiated by the carotid sinus reflex. Derom therefore restricts the outflow of preganglionic fibres to the inferior extremity to these three spinal roots, although he did not determine whether it was necessary to cut all of these. His results fall within the major outflow we describe, but his method is probably not sensitive enough to define the limits of the total outflow where the roots afford only weak contributions.

An emergence of vasomotor fibres from the lowest lumbar segments to the inferior extremity is suggested by the observation of Oughterson, Harvey and Richter¹². They found that ligation of the femoral artery in the dog caused a fall in skin temperature which remained for several hours and was followed by a spontaneous rapid rise in temperature, indicating the opening up of collateral circulation. Since removal of the sympathetic supply hastened the return of skin temperature to previous levels, they concluded that vasoconstriction is a factor in the initial fall and that a procedure like transection of the cord, which also offsets the temperature drop, is a good test for the detection of the level of exit from the cord of vasoconstrictor pathways. Utilizing this test Oughterson, Harvey and Richter found that transection of the spinal cord as low as L6, three to four hours after ligation, was followed by vasodilatation in the lower extremity. Presumably section at this level severed vasoconstrictor pathways descending within the cord to emerge below L6. There are many factors, however, entering into this indirect method of estimation. One misses, therefore, the decisive indication of validity that would have been afforded by the comparison in each animal of the simultaneous changes taking place in an unligated control limb with those in the ligated limb after cord transection.

The upper level of the outflow to the superior extremity in man is of special significance in planning any operation designed to divide only preganglionic fibres. It is particularly unfortunate that precisely here there should be lack of exact anatomical knowledge. The findings in the monkey as outlined in the present study do not admit the acceptance of preganglionic fibres destined for the upper limb emerging from the spinal cord above T4. The results are in close accordance with the analyses of sudomotor and vasomotor pathways to the upper limb as stated by Langley, and Bayliss and Bradford. It is worthy of note that Sherrington¹⁴ in his studies of the lumbosacral and brachial plexuses of the monkey did not mention any vascular changes in the upper extremity upon stimulation of the ventral roots of T1, T2 or T3 although special attention was paid in these experiments to sympathetic effects. Excitation of T1 in addition to movements of the fingers and wrist produced *only* dilatation of the pupil. Excitation of T2 and T3 in addition to somatic motor responses produced dilatation of the pupil, opening of the palpebral fissure, erection of hair on the scalp, erection and paling

of the pinna, and vasoconstriction in the lateral lobe and one half of the isthmus of the thyroid gland.

Kuntz, Alexander and Furculo⁵ however believe that preganglionic neurons involved in the sympathetic innervation of the upper extremity are located in the upper thoracic region of the spinal cord, beginning with the first thoracic segment and extending downwards at least as far as the third or fourth segment. They base their belief on experimental histological evidence and also upon observations following stimulation of the ventral roots of these spinal nerves. In cats unilateral section of the roots of T1 was followed two to three weeks later by complete degeneration not only of the preganglionic fibres entering the stellate ganglion through the white ramus of this nerve, but also of the major portion of the inter-cellular fibre-complex in the portion of the ganglion adjacent to this ramus, and they add "the number of ganglion cells with which preganglionic components of the first thoracic nerve effect synaptic connections obviously is large. The *distribution** within the ganglion of the axons arising from these ganglion cells, furthermore, indicates that many of them enter gray rami which join the nerves which make up the brachial plexus." Such evidence is however inconclusive in view of the present inadequate knowledge in regard to the location of the post-ganglionic cells related to the limbs.

Kuntz, Alexander and Furculo⁵ however report sweating in the pads of the fore-foot on stimulation of the ventral root of either T1, T2 or T3, and also a constriction of the ulnar artery when exposed in a distal part of the extremity. These findings are entirely at variance with previous observations and we are inclined to believe that there may have been some unobserved spread of stimulating current, a complicating factor which in such experiments is one of the most difficult to eliminate.

Smithwick¹⁵, from his extensive clinical experience, believes that in man there may be sometimes a sympathetic outflow from the second and third thoracic segments to the upper extremity. He claims that, in the treatment of vascular spasm of the hand, in addition to section of the sympathetic chain below the 3rd thoracic ganglion, division of the spinal roots of T2 and T3 should be performed, holding that section of these roots instead of their rami (as in Telford's operation) offers surer prevention of regeneration. The findings in the monkey recorded above cannot of course be transferred directly to man, but the fact that the outflows of preganglionic sympathetic fibres to the extremities in cat, dog, and monkey, are so nearly the same, implies a similar arrangement in man, and the more restricted outflow in the monkey would lead one to expect a narrower rather than a more extensive outflow in man.

Crossed pathways between the sympathetic chains certainly exist as an anatomical possibility. Langley has shown that in the cat, one at least of the sacral ganglia is connected by transverse strands with the corresponding

* The italics are ours.

ganglion of the opposite side, and sometimes the first sacral ganglia on the two sides form one fused median ganglion. The transverse connections, according to Langley, serve largely the purpose of connecting the lumbar white rami on each side with both sides of the pelvic viscera, but he also found some evidence of crossed pathways to the lower limb. By stimulating the peripheral cut end of the sympathetic chain above the sixth lumbar ganglion, he observed slight sweating on the opposite foot on a few occasions, and concluded "that either a few preganglionic fibres cross to the ganglia of the opposite side, or some of the post-ganglionic fibres of one side run to the opposite grey rami."¹⁰ The question of such crossing has gained new significance in view of the clinical finding that after *unilateral* lumbar sympathectomy *both* lower extremities show an initial marked rise in skin temperature. The results of the present experiments show that neither in the cat nor in the monkey can such pathways be detected. The explanation of the vasodilatation in the contralateral limb following unilateral sympathectomy probably then has some other basis.

The presence of B fibres in the sympathetic outflow to the limb in mammals has not hitherto been conclusively proven. Erlanger and Gasser⁵ had shown that in the frog the sciatic B potentials must pass through the grey rami. On stimulating the grey rami in cats and dogs all the successful preparations gave C waves in the femoral nerve, but Erlanger goes on to say in a "relatively few . . . an elevation with the threshold and rate of conduction definitely within the B range" (10.5 m. per sec.) was also detected. However, if the recording electrodes were placed on branches of the "sciatic plexus" B waves could not be obtained. The existence of B as well as C potentials, amongst the responses, here shown to be autonomic by their disappearance after nicotine, is of considerable interest. The presence of the corresponding B fibres implies the possibility of control over effectors differing in function and distinct from those supplied by fibres of the C group. The number of B and C fibres contributed by each root would then determine the possible extent of its control over the various types of sympathetic responses. This is but one tentative interpretation. If the scarcity of B waves in the responses from the radial nerve, as compared with those from the ulnar and median, turns out to be an accurate picture one wonders whether it might be related significantly to a difference in anatomical distribution. The ulnar and median nerves supply the palmar surface of the hand, an area which sweats profusely under emotional stress. The cutaneous distribution of the radial nerve on the other hand is confined to the back of the arm and forearm and dorsum of the hand, where emotional sweating is negligible. May there not be some correlation between the autonomic B fibres in the ulnar and median and the sudomotor function of these nerves? The data are not extensive enough to answer the question with assurance. The significance of the finding that B waves form only a decidedly minor part of the sympathetic potentials in the monkey as compared to the cat awaits final interpretation of the function of autonomic B fibres.

SUMMARY

1. The sympathetic preganglionic outflow from the spinal cord to the limbs has been re-investigated by recording action potentials in the peripheral nerves following stimulation of ventral roots.

2. In the cat the outflow to the lower extremity extends from T11-L4, with the major contribution from T13-L13 (inclusive), whereas in the Rhesus monkey (*Macaca mulatta*) it extends from T12-L3, with the major contribution from L1-L3 (inclusive). Thus 7 spinal roots in the cat participate in the outflow to the lower limb, whereas only 4 roots contribute in the monkey.

3. The sympathetic preganglionic outflow to the upper extremity extends in the Rhesus monkey (*Macaca mulatta*) from T4-T8 (inclusive) with the major outflow from T5, T6, and T7. This outflow is also more restricted than that observed in the cat and dog by previous workers.

4. There is no definite difference between the extent of total outflows to the median, ulnar and radial nerves, nor between the outflows to the medial and lateral popliteal branches of the sciatic.

5. Variations in the fixation of the lumbo-sacral and brachial plexuses were not associated with any clear differences in the preganglionic outflows to the limbs.

6. Crossed pathways, between the lumbo-sacral sympathetic chains, of fibres destined for the opposite sciatic nerve were not demonstrated in either the cat or the monkey.

7. Large C waves were recorded in both "animals." B waves were recorded in the cat, but were only occasionally seen in the monkey.

8. Nicotine injected intravenously in suitable doses abolished both B and C waves, thus establishing their autonomic nature.

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RECOVERY OF RESPONSIVENESS IN MOTOR AND SENSORY FIBERS DURING THE RELATIVE REFRACTORY PERIOD

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IN THE course of experiments designed to show whether fatigability is different in sensory and motor nerve fibers, it appeared necessary to determine whether there were differences between the curves of recovery during the relative refractory state *without fatigue*.

METHOD

Twenty-five bullfrogs and 10 cats were used for the experiments. The bullfrogs were generally pithed and the 8th and 9th spinal roots cut at their entrance into the cord. The tibial and peroneal nerves were cut at the knee. In some cases the entire sciatic nerve with its spinal roots was excised and set up in a moist chamber; in others the nerves were left *in situ*. In 7 experiments recovery was observed also in the isolated roots. Most of the cats were spinal animals; a few were narcotized with dial. Their 6th and 7th lumbar roots were ligated and cut centrally, and here too the popliteal nerves were cut.

The conditioning and the testing shocks were delivered by two Harvard coils, with the secondaries in series. The primary circuits were opened by means of two keys on a Lucas pendulum, the circle of which was divided into quarter degrees. The speed of the pendulum, taken as constant for the small intervals used, was $1\frac{1}{2}$ degrees per msec. The limit of accuracy in setting the pendulum was about 0.04 msec. As we were interested chiefly in the recovery of responsiveness (height of spike) and not of excitability, we used strong testing stimuli, generally three or five times stronger than just maximal. In a few experiments in which we tested the same nerve with stimuli three, four and eight times maximal, there was almost no change in the shape of the curve. Thus we were assured that the curves obtained did not depend upon the number of fibers excited but represented the average height of the potentials in *all* the fibers at each interval. Choosing the right strength of conditioning stimulus for the sensory fibers occasionally offered difficulty. Sometimes the α wave of the second response at short intervals overlapped the β wave following the testing stimulus. Therefore, it was important that the β wave should have a constant height in order to make accurate measurement of the second α response possible. That is, the testing stimulus had to be strong enough to elicit a maximal β response. Otherwise at short intervals summation of the second stimulus with the first one might have excited β fibers that had not responded to the first stimulus alone, thus making the second α response appear higher than it actually was. Figure 1, a series of original records from a sensory root, is a good example of how necessary it was that the β fibers be stimulated maximally by the conditioning shock. On the other hand, if the conditioning stimulus was too strong there was danger of repetitive response of the α fibers. Sometimes in sensory roots of the cat (L6 and L7) there was a poor separation of the α and β spikes so that it was difficult to measure the height of the first. The pictures of double responses of sensory fibers were not always so easily interpreted as those in Fig. 3. Frequently if the recording electrodes were placed close to the cut end of the root in the cat, very small potentials were obtained; but the responses became normal when the electrodes were shifted a few millimeters peripherally. It was not determined whether recovery also was impaired near the cross-section of the roots, but this seems highly probable. Hence in placing the electrodes on the roots too near their cut ends, there was a source of error hard to avoid. Therefore experiments on roots giving strong action potentials seemed far more reliable than those on roots with poor potentials. Generally the stimuli were applied either at the central or at the peripheral end of the preparation, the recording electrodes being placed at the opposite end.

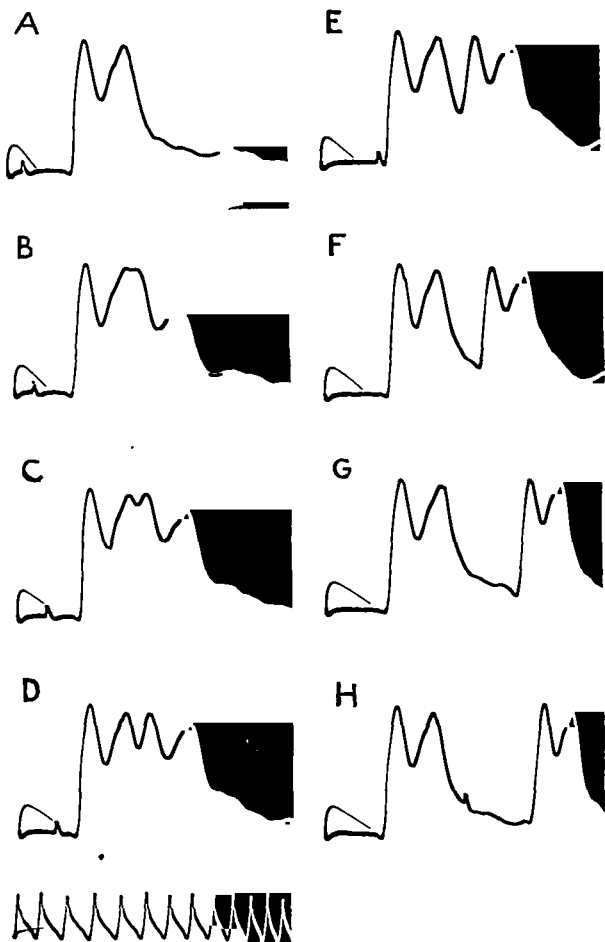


FIG. 1. Action potentials recorded from the 9th sensory root. Stimulation of the sciatic nerve *in situ*. Time intervals between conditioning and testing stimuli in successive pictures: 1.1, 1.3, 1.7, 2.1, 2.7, 3.7, 5.1 and 6.3 msec. Time: milliseconds. (Bullfrog 29; 7-20-40, Temp. 27°C.)

The action potentials were led through a Grass condenser-coupled amplifier and registered on a cathode ray oscillograph adjusted for a single sweep controlled by the Lucas pendulum to start simultaneously with the conditioning shock. For both stimulation and recording Ag electrodes were used.

RESULTS

Frog experiments. The shortest absolute refractory state observed lay between 0.6 and 0.8 msec. (Temp. 25 to 28°C.). In the nerves *in situ* these abnormally long periods probably were due to the inevitable impairment of

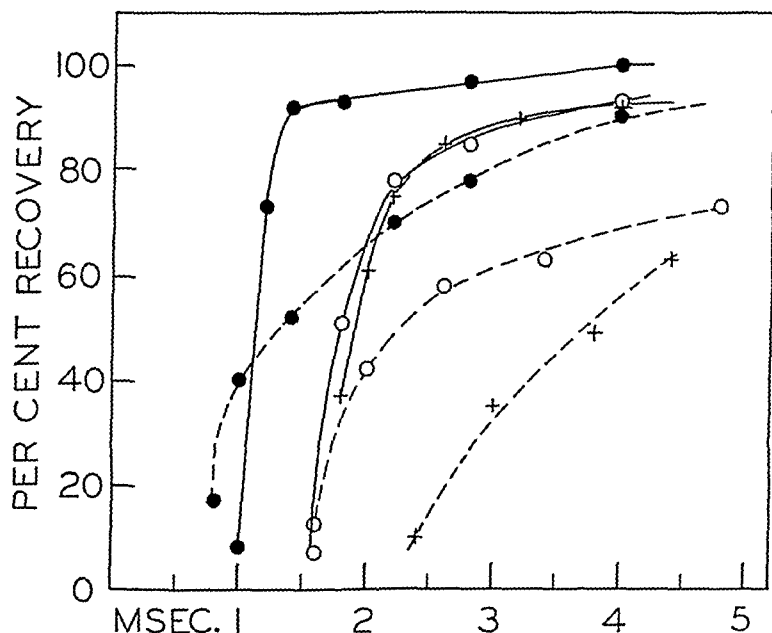


FIG. 2. Recovery of height of α potentials during the relative refractory period in motor (solid lines) and sensory (broken lines) fibers in three bullfrog experiments. Dots: recorded from the roots, nerve *in situ* (Bullfrog 22; 5-28-40). Circles: recorded from the roots, nerve *in situ*; the sympathetic rami to the lumbar plexus had been cut (Bullfrog 18; 5-7-40). Crosses: recorded from sciatic trunk, roots stimulated, excised nerve (Bullfrog 15; 4-27-40). Abscissae: interval between conditioning and testing shocks. Ordinates: height of conditioned response in per cent of height of first response.

circulation arising from pithing and the preparation of the roots. In some cases the absolute refractory period was even as long as 2 msec. Often, but not invariably, the dorsal roots showed the longer periods (cf. Fig. 2).

The curves showing recovery of responsiveness in motor fibers varied from frog to frog. Most of them showed a steep initial rise, attaining about 80 to 95 per cent recovery within 0.5 msec. after the end of the absolute refractory period. This was followed by a slow increase to 100 per cent, the curves in general being knee-shaped (see Fig. 2, motor curve indicated by dots). In some cases this knee did not appear so sharp. If the experimental

points had been closer together these curves might have approached the others in form (Fig. 2, motor curve indicated by circles). In a few cases where the roots had been stretched, complete recovery was much slower; the initial rise of the curve showed typical steepness, but the knee occurred when recovery was not so far advanced as usual (in one case only 50 per cent).

Recovery of responsiveness in the sensory fibers was always much slower and the knee-shape so often found in motor curves was absent. Ninety per cent recovery was obtained on an average within 0.8 msec after the absolute re-

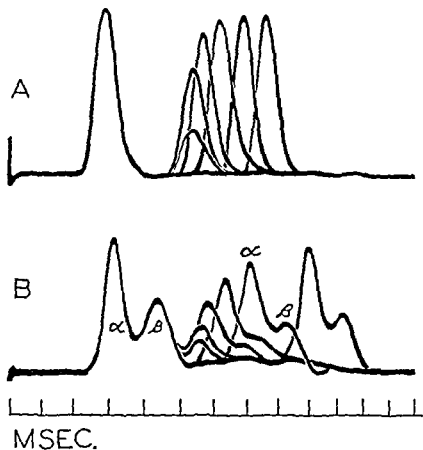


FIG. 3 Action potentials of motor fibers (A) and sensory fibers (B) recorded from the frog roots. The roots were stimulated with two maximal stimuli, one at intervals of 1.4, 1.6, 2.0, 2.6, 3.6, 4.4 msec. Successive pictures have been superimposed by projection so that the first responses are coincident (Bullfrog 10, 5 5-40)

fractory period in the motor curves and within 4 msec. in the sensory. A survey of motor and sensory curves of all the frogs used shows that in spite of the great individual differences there was a clear segregation of sensory curves from motor. The slowest motor curves were approximately coincident with the fastest sensory ones. Yet there generally was a certain relation between the rate of recovery in these two sets of fibers, a relatively quick motor recovery being associated with a relatively less slow recovery in the sensory fibers (cf. Fig. 2). Among about 40 pairs of motor and sensory roots, only two were found having identical curves; in no case did the sensory root recover more quickly than the motor. Figure 3 further illustrates this marked

functional difference between motor and sensory α fibers. Although accurate measurements of the heights of the β waves could not be made, the curves in *B* (Fig. 3) indicate that recovery in these fibers was still slower than in the sensory α fibers.

In many cases repeated observations were made on the same preparation. When there was evidence of deterioration of the nerve, such as drying, successive curves were found to become slower (cf. Fig. 4). However, later curves taken on nerves which were apparently still in good condition showed a

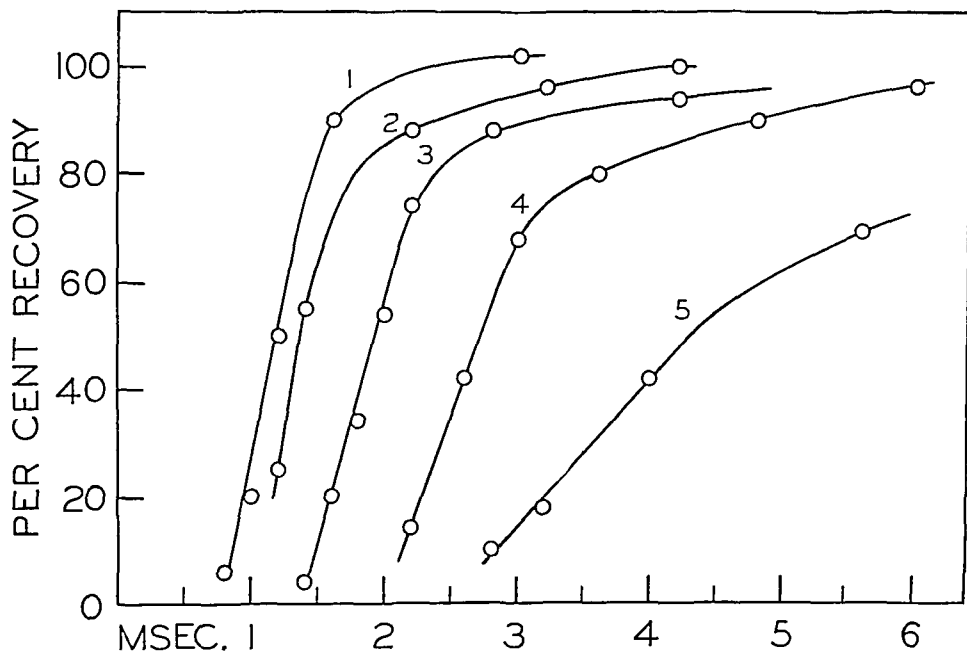


FIG. 4. Effect of gradual drying-out on recovery of height of spike during relative refractory state. Pithed leopard frog in moist chamber, recording from tibial nerve *in situ*. Double stimuli applied to sciatic high in the hip. Curves 1 to 5 taken at 2:42, 3:05, 3:45, 4:08 and 4:22. At 4:25 nerve found very dry. (5-22-40.)

tendency to become faster. These observations confirm similar findings of Graham (1934). In Fig. 6 a curve taken from a sensory root 29 hours after excision showed that recovery was faster than on the previous day.

Measurements on recovery of conduction velocity (calculated from the time interval between the start of the testing stimulus and the foot of the recorded spike) were made on nerves of 8 bullfrogs. This method was used in spite of the recognized inaccuracy involved (Graham and Lorente de Nó, 1938). It is remarkable that no constant difference between recovery of conduction velocity in motor and in sensory fibers was found. The motor and sensory curves were either identical (cf. Fig. 5) or showed only slight variations in either direction. At the start of the relative refractory period,

velocity was generally reduced to about 60 per cent. The greatest reduction seen was to 55 per cent, at an interval of 1 msec. Recovery of velocity was faster than that of size of response in the sensory fibers but slower than recovery of responsiveness in the motor (cf. Fig. 5).

To determine whether slowness of recovery of responsiveness was an inherent characteristic of the dorsal root fibers, recovery curves were taken for the excised 9th sensory and motor roots of 6 bullfrogs, the spinal ganglia having been cut away. Unexpectedly, these curves were found to be similar

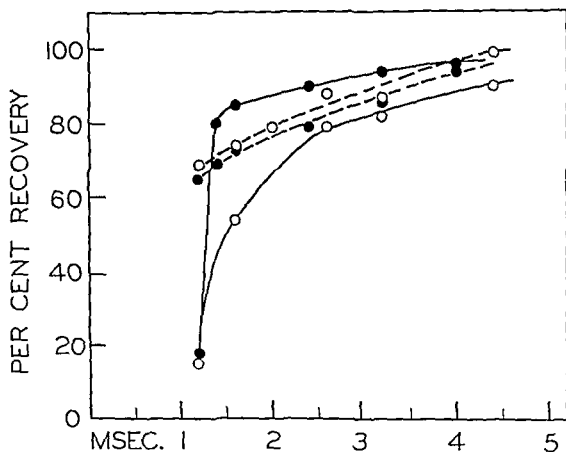


FIG 5 Recovery of responsiveness (solid lines) and conduction velocity (broken lines) in sensory (circles) and motor (dots) fibers, recorded from the sciatic trunk. Excised nerve in moist chamber. Abscissae: interval between conditioning and testing shocks. Ordinates: height of conditioned response in per cent of height of first response, and conduction velocity of conditioned impulse in per cent of velocity of first impulse (Bullfrog 15, 4-27-40)

and in some cases even identical. Recovery of responsiveness of sensory fibers was much faster in the cut root than when these fibers were tested in either direction as part of the root-nerve preparation. Figure 6 illustrates this behavior. Later, stimulating the 9th spinal nerve 2 to 3 mm. peripheral to the spinal ganglion and recording from the central end of the dorsal root, we found the typical slow recovery curve, whereas by shifting the stimulating electrodes to a point the same distance central to the ganglion we obtained the quick curve (two experiments). Figures 4 and 6 seem to show a recovery of spike height to more than 100 per cent. As no supernormal phase ever has been observed in recovery of responsiveness (Graham, 1934) this

overshooting may be due to error in method. Often recovery curves of cut ventral roots showed no difference from those of the root-nerve preparations. However, when the latter had the sharp knee-shape, the curves of the same roots when isolated were somewhat slower (rounded off).

As sensory fibers in toads contain only $\frac{1}{6}$ of the acetylcholine (ACh) concentration found in motor fibers (Chang, Hsieh, Lee, Li, and Lim, 1939) it seemed interesting to determine whether ACh has any influence on the re-

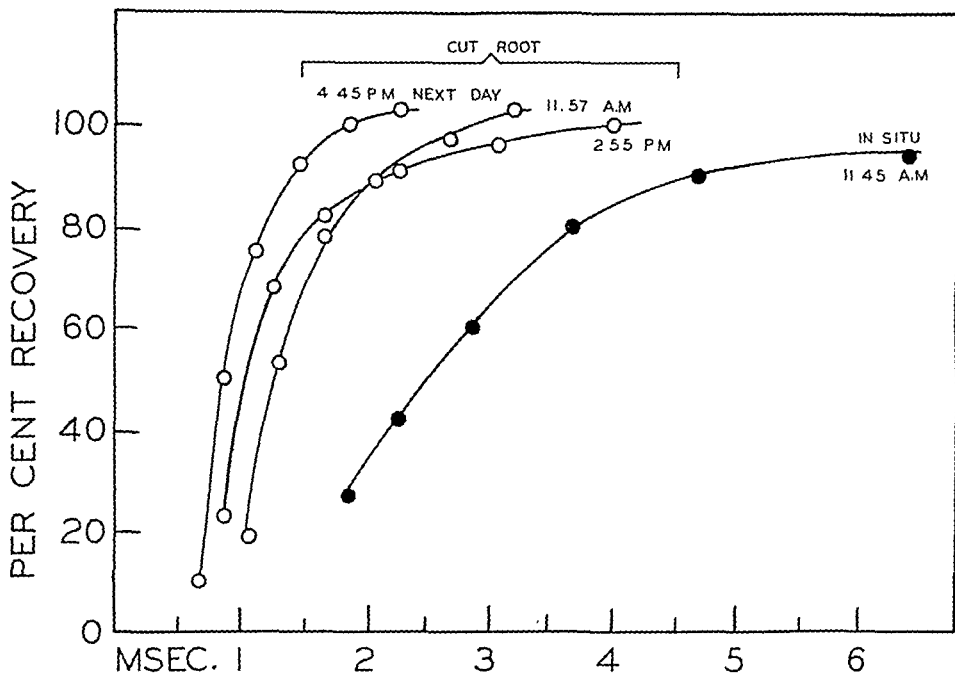


FIG. 6. Recovery of height of spike in a dorsal root during relative refractory period. Dots: stimulating electrodes on sciatic nerve *in situ*. Circles: experiments on the same root excised without the ganglion. (Bullfrog 31; 7-20-40.)

covery of responsiveness. Sciatic nerves of some leopard frogs and spinal roots of 6 bullfrogs were excised. One of each pair was put into Ringer's solution; the other for $\frac{1}{2}$ to 1 hour into a solution of eserine sulfate 1:50,000 and afterwards for 1 hour or more into ACh 1:25,000 to 1:100,000. When these nerves were tested with double stimuli, no characteristic difference between recovery curves of Ringer and of ACh nerves could be seen.

Cat experiments. The characteristic difference observed between recovery of responsiveness in motor and in sensory fibers in the bullfrog did not appear in the cat. In some cases recovery in both roots was equal, in others it was quicker either in the motor or in the sensory (cf. Fig. 7). Although the shapes of the curves varied remarkably, the knee-shape, characteristic of the motor fibers in the bullfrog, was seldom seen (cf. curves indicated with circles in Fig. 7). In general, recovery in these cat experiments was markedly slower

than in the bullfrog *motor* fibers; in fact the cat curves were very similar to the curves of the *sensory* fibers in the bullfrog. In a few experiments on cut roots recovery was found to be quicker than in the root-nerve preparation; in others it was about the same. The number of experiments was too small to give conclusive results. The relatively low temperature (about 31°C.) in the moist chamber into which the dorsal wound had been transformed may account for the relative slowness of the cat curves.

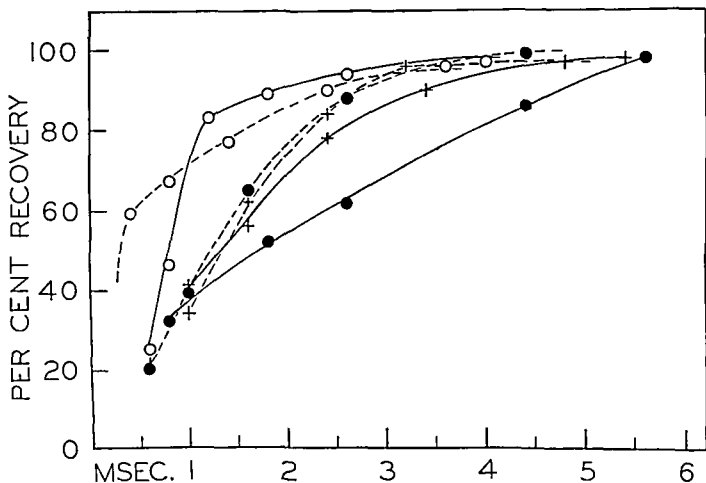


FIG 7 Recovery of height of α potentials during the relative refractory period in ventral (solid lines) and dorsal (broken lines) roots in three pairs of cat nerves. Stimulating sciatic nerve *in situ*, recording from lumbar roots. Circles: Cat 2, L7, 5-18 40, crosses: Cat 9, L6, dots: Cat 9, L7, 7-15-40.

The absolute refractory period on the whole was shorter than in the bullfrog experiments. Often a second response was seen at an interval of 0.6 msec. and in a few cases even at 0.4 msec.

In 5 experiments recovery of conduction velocity was measured by the method described in the former section. These curves closely paralleled the curves of recovery of height. The greatest reduction in velocity observed was to 40 per cent at an interval of 0.6 msec.

In cat fibers also ACh was found to have no observable effect upon recovery of responsiveness.

DISCUSSION

The outstanding result of these experiments is the characteristic difference between the curves of recovery of responsiveness in motor and in

sensory fibers in root-nerve preparations of the bullfrog. As the experiments with cut roots demonstrate that the slow recovery is not an inherent quality of the sensory fibers, the question arises as to the cause of the observed difference. Since this same difference was found in experiments in which the stimuli were applied either to the root or to the peripheral end of the nerve, it cannot be due to any influence set up in the nerve trunk. It appears that the *spinal ganglion* must be the source. That the difference arises only when the impulse passes through the ganglion, its presence alone not being sufficient, is concluded from an experiment in which the root had been stimulated centrally from the ganglion before and after cutting the ganglion away, the same fast curve being obtained in both cases from the root.

The phenomenon is independent of the blood supply to the ganglion, since it was observed in root-nerve preparations both excised and *in situ*.

The simplest explanation of the phenomenon would be the assumption of a temporal dispersion of the α spikes during the passage through the ganglion, reducing the height of the spike by broadening its base. Erlanger and Blair (1938) studying single impulses found it not "possible to substantiate a lag in the propagation through the ganglion." It still seems possible, however, that a *conditioned* impulse travelling more slowly might be retarded. If this retardation were different for different fibers, it might account for the observed effect. The question whether or not this is the case might be checked by comparing the areas under the conditioned spikes at given intervals in experiments in which the root spikes were recorded when stimulation was applied both central and peripheral to the ganglion. Such a comparison is possible only if the "preganglionic" stimulation is applied fairly close to the ganglion; otherwise, a long conduction path allows the α and β waves of the first impulse to separate, making it impossible to see the actual initial rise of the conditioned α wave (cf. Fig. 1). With pre-ganglionic stimuli close to the ganglion, the rise of the conditioned response is clean. If the reduction in height of the spike after passing the ganglion is purely a matter of retardation, the presence of the β wave in the conditioned response should not interfere with the comparison. This comparison was made for three pairs of spikes. In each case the area under the spike that had passed the ganglion was found to be decisively smaller than under the other. The fact that this characteristic difference between motor and sensory roots after stimulation of the mixed nerve does not appear in the cat preparation is another point against the idea of temporal dispersion.

Thus, in spite of the fact that the explanation by temporal dispersion would be by far the simplest, it does not seem acceptable. Even if one might assume decremental conduction in the region of the ganglion, one would expect the impulse to return to normal as soon as it had passed the ganglion.¹

¹ Dr. R. W. Gerard has suggested to us the possibility that slow recovery of excitability in the ganglion might cause complete blocking of impulses in some of the fibers with a consequent reduction in the total response recorded beyond the ganglion. Our results may well be interpreted on this basis.

It appears necessary to assume that the passage of the impulse through the ganglion really modifies the responsiveness of the fibers beyond the ganglion during the relative refractory state by causing the spread of an influence along the fibers. Such an influence might be either electrotonic or chemical. In the latter case the chemical process of which the spike potential is an indicator would be modified during and after passage through the ganglion. Since in a series of side experiments we could detect no influence of ACh on recovery of responsiveness in isolated roots, it does not seem probable that this substance is the cause of the phenomenon.

The question as to how far the presence of the ganglion cell influences the nerve fiber arising from it has been frequently discussed ever since Fröhlich and Loewi (1908) postulated in a cephalopod the "feeding" of a nerve by its ganglion with a substance necessary for nerve excitability. On the basis of our present knowledge it is not profitable to discuss this question further.

In the course of the experiments described in this paper, recovery of excitability was studied only occasionally, but our experiments confirmed Graham and Lorente de Nó's (1938) results showing that recovery of conduction velocity occurs more promptly than that of excitability and that there are no late changes in velocity and in responsiveness corresponding to the supernormal and subnormal periods of excitability. The fact that conduction velocity recovers at the same rate in sensory and in motor fibers, in spite of the marked difference in recovery of responsiveness, may be of interest for the theory of propagation of the nervous impulse. Erlanger and Blair (1938) found that the sensory fibers in the bullfrog's sciatic are much more excitable than the motor and yet they both conduct at about the same velocity. They suggest that the "tendency the high excitability must exert towards hurrying propagation might be counterbalanced by the increased number of restimulations needed per unit of distance traversed" (shorter segments between the nodes of Ranvier). If conduction depends on restimulation by eddy currents reaching threshold strength for the adjoining segment of the fiber, conduction velocity must depend also on the time elapsing between the start of the local potential and the moment when it reaches threshold strength for the next segment. This time being longer in potentials of low voltage, recovery of conduction velocity ought to be slower in sensory fibers after passage through the ganglion than in motor fibers. The fact that this is not the case may be due to a counterbalance exerted by the higher excitability of the sensory fibers. The absolute magnitude of motor and sensory action potentials was not measured in our experiments. Yet the fact that when the same amplification was used the potentials of motor fibers were always much greater than those of sensory α fibers might indicate that motor fiber potentials are of higher voltage than sensory ones. This is in accord with Erlanger and Blair's (1938) observation that in the bullfrog the demarcation potential of the sensory root is lower, by roughly a third, than that of the motor root. A relatively lower voltage in sensory fiber potentials

should also be kept in mind as a possible counterbalance to the influence higher excitability exerts on conduction velocity.

In 1937 Gasser discussed whether one might suppose that the relative refractory period and the subnormal period which occurs during the positive after-potential are one continuous event into which is interpolated a super-normal period associated with the negative after-potential. Gasser at that time did not accept this point of view, because it was held that, whereas the spike remained full sized during the subnormal period, it was *undersized* during the relative refractory period. In the following year it was shown by Graham and Lorente de Nó that recovery of responsiveness and of excitability during the relative refractory period occur at quite different rates, responsiveness being fully recovered at a time at which excitability still is much reduced. In many of our experiments on bullfrog and leopard frog α fibers the correctness of this statement has been fully confirmed, and Grundfest (1939) found the same to be true for β fibers. According to these results Gasser's suggestion is no longer to be rejected (as it was by himself in 1937). No fact is known incompatible with the view that the subnormal period is a late continuation of the relative refractory period but separated from it by a phase of supernormal excitability.

SUMMARY

Recovery of responsiveness (height of spike) and of conduction velocity in motor and sensory fibers during the relative refractory state was studied in bullfrogs and cats. A Lucas pendulum was used for timing the stimuli and a cathode ray oscillograph for recording.

In bullfrogs recovery of responsiveness in the sensory fibers including the spinal ganglion was always much slower than in the motor fibers. The sensory and motor curves attained 90 per cent recovery within about 0.8 and 4.0 msec. respectively after the end of absolute refractoriness. In dorsal roots *without* ganglia, however, responsiveness recovered at the same rate as in ventral roots.

The passage of the impulse through the ganglion seems to modify the responsiveness of the fibers beyond the ganglion.

In cats recovery of responsiveness was slower in general (average temp. in moist chamber 31°C.) and no influence of the spinal ganglion could be observed.

In both bullfrogs and cats conduction velocity recovered at the same rate in sensory fibers including the spinal ganglion and in motor fibers.

Acetylcholine was found to have no observable effect upon recovery of responsiveness.

It is suggested that the subnormal period is a late continuation of the relative refractory period (cf. Gasser, 1937).

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TECHNIQUE AND EVALUATION OF THE ELECTROENCEPHALOGRAM*

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INTRODUCTION

EVER SINCE Berger in 1929 first described the human electroencephalogram (EEG), investigators have attempted to describe and interpret these records. Our experience is based on 5 years of standardized recording of more than 1100 individuals, both normal and abnormal, and it now seems worth while to set forth our own attempts at a systematic evaluation.

This paper deals with four objectives: (i) A description of standardized technique, which is the basis of other papers already published and in preparation. (ii) A description and evaluation of some of the previous and current methods of measurement. (iii) A classification of fundamental types of EEG patterns useful for description and correlation. (iv) A system of evaluation as to degree of normality and abnormality.

Selection of individuals. The individuals, 15 or more years of age, whose EEG's have been recorded, have been classified with respect to their "normality." We fully realize that there is a large subjective factor which cannot be excluded when evaluating human beings and their behavior. Nevertheless a person's medical history and his behavior under different conditions and in different environments, judged by several individuals who know the person, seems to us to constitute the soundest and most practical basis for classification. The definitions of the various categories are as follows:

1. *Selected normal.* On these individuals we have extensive and reliable medical and social history. Their medical history is within normal limits and reveals only the ordinary childhood diseases without unusual sequelae. There is no history of even one convulsion, regardless of cause. There have been no psychiatric problems necessitating care, nor have there been questionable relationships with others. Their behavior has been normal and wholesome at home, at school, at work and at play.

* Although the editorial policy of the *Journal* has been never to accept papers dealing solely with technique, a unique exception has been made in this case. The Editors have desired to assist in making this technique available as soon as possible since its application at the moment to problems of aviation medicine seems pressing. Ed.

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2. *Presumably normal.* Information on this group is less complete, but, as far as it goes, indicates that the individuals are normal. The group includes many college and medical students whose school, social and home life is known to some extent. The group thus differs from the usual so-called "control" group.

3 *Unknown.* The group includes casual visitors, friends of friends, employees or students not well known to us.

4. *Abnormal. A. Outside hospital.* Persons belonging to this group are those who have needed psychiatric attention and those whose inability to adapt to ordinary circumstances is obvious to their friends or associates. Under this heading are epileptics or people whose history reveals one or more convulsions, even though they are apparently normal.

B. Patients in mental hospital. This group includes mostly patients in mental hospitals [McLean Hospital (private patients, unselected)]. Aside from these, there are a few cases from general hospitals whose condition involves psychiatric or neurological problems.

PROCEDURE

In the first year many experiments were performed in attempts to modify the EEG. The results showed that it was important that the subject be at ease and free from apprehension in relation not only to the procedure, but also to the person who records him. It was found that a light-proof or sound-proof room is not conducive to putting the person at his ease. If the eyes are open, it is not only light which modifies or reduces some of the rhythms of the EEG, but the fluctuation of attention and the adjustment of "psychological set" (Davis, P. A., 1939; Davis, H. and Davis, P. A., 1939) which tend to alter the stability of the record. Our standard procedure, which fulfills the optimum conditions for stability, requires that the person be comfortably relaxed but thoroughly awake, with his eyes closed, on a bed facing away from the source of light in a diffusely lighted room. The subject should be clearly in view at all times if it is not possible for him to be in the room with the recorder. It is essential that conversation or whispering between recorder, another doctor or nurses be strictly avoided during the procedure so that recorder and subject can maintain a friendly rapport throughout.¹

Apparatus The ink-writing oscillograph with its amplifiers and broad-band filters, built by Mr Albert M Grass for the study of the EEG, has become standardized for routine recording since we began our studies. The speed of the tape is 3 cm per sec. The standard sensitivity is 1 cm for 100 μ V. The frequency characteristic of the recording system is

¹ Persons being recorded under standard conditions should be known to be free of drugs, even as common a one as aspirin, for several days before recording. For instance, one of our patients showed an elevated blood-bromide level for 3 weeks after cessation of medication with sodium bromide and the EEG became normal only after the blood-bromide concentration had fallen to the normal range. And it is not only the drug but the fact that the condition of the person is such that any medication is required that implies that he is not in perfectly normal condition. Throughout this paper all measurement data on both normal and abnormal people are based on subjects free of medication and recorded under standard conditions.

flat from 3 to 65 cycles. The upper limit is often deliberately reduced by a "muscle filter," which is built into the power amplifier, to 50 cycles so that extraneous electrical disturbances and muscle potentials are reduced to a minimum. A time-constant of the system of 0.15 sec.² is found satisfactory for standard records.

Tuned electrical filters have been built so that they can be included or excluded in the circuit as desired. The frequency-bands of these filters are broad,³ and overlap considerably. The peaks of the frequency-bands have been arranged fairly close together and extend from 3 to 35 cycles. Thus a 10-cycle filter has a broad peak at 10 cycles. It will pass 9 and 8 and even 7 cycles on one side, and 11, 12, and 13 cycles on the other side, although the amplitudes of the waves will be somewhat less. Up to and through 1939, the filters Mr. Grass has built and which we have used are tuned to 3, 5, 7, 10, 14, 17, 23, 28, and 35 cycles.

Amplification is increased when the filters are employed, so that waves at the frequency to which the filter is tuned appear at approximately double the amplitude that they show

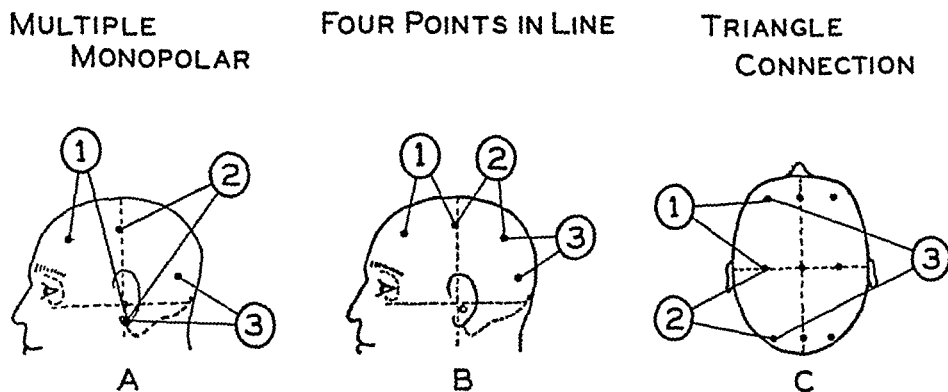


FIG. 1. Placements on head representing different techniques of recording. The numbers represent the amplifiers.

in the standard unfiltered record. When the filters are switched into the circuit, standard amplification for them is obtained by decreasing the attenuation four steps on the Grass amplifiers.

At present we are using a 3-pen recorder, with broad-band filters and perfectly matched amplifiers. The amplifiers are provided with balanced push-pull input stages which are not directly connected to ground. Therefore the three systems are non-interfering and completely independent of one another.

Multiple "monopolar" recording. Unless otherwise specified we shall deal in this paper with multiple monopolar records. We have regularly used the lobes of the ear as our reference point, as shown in Fig. 1A. Specially constructed ear-clip electrodes are very conveniently applied. Control experiments show that the ear is electrically much less active than points on the cranium. The mastoid process has been found to be unsatisfactory because of its proximity to the temporal region.

² When a constant potential difference is applied to the input leads of a condenser-coupled amplifier system the recording pen registers a quick deflection followed by a relatively slow return to the base-line. The "half-time constant" of the system is the time required for the pen to return halfway. The "time-constant," more generally employed by electrical engineers, is the time required for it to return to $1/e = 1/2.718 = 0.368$ of its initial deflection. The longer the time-constant the lower is the frequency of an alternating current that will be recorded without attenuation.

³ The breadth of the band is conveniently defined in terms of the frequency whose voltage is attenuated to one-half of the voltage that is delivered at the resonant frequency at the center of the band. For our present filters the ratio of frequency for 50-per-cent attenuation to the resonant frequency is 1.55, i.e., the filter tuned to 10.1 cycles attenuates by 50 per cent the waves at 15.7 or 6.4 cycles. The breadth of band is the same for all

Table 1

First, unfiltered at standard, then at increased sensitivity Second, all filters at their standard sensitivity			
Nine-point routine Multiple monopolar recording			
Simultaneous recording of one area on the two sides of the head			
	A	B	C
Line 1	Left occipital	Left precentral	Left frontal
Line 2	Right occipital	Right precentral	Right frontal
Line 3	Mid occipital	Mid precentral	Mid frontal
Simultaneous recording of three areas on each side of the head			
	D (left side)	E (right side)	
Line 1	Left frontal	Right frontal	
Line 2	Left precentral	Right precentral	
Line 3	Left occipital	Right occipital	
Bipolar record by "triangle" connection, if indicated			
Line 1	Left frontal to left precentral	Right frontal to right precentral	
Line 2	Left precentral to left occiput	Right precentral to right occiput	
Line 3	Left occiput to left frontal	Right occiput to right frontal	
Bipolar record by four points in line			
Line 1	Left frontal to left precentral	Right frontal to right precentral	
Line 2	Left precentral to left parietal	Right precentral to right parietal	
Line 3	Left parietal to left occiput	Right parietal to right occiput	

frequencies except the lowest which is very broadly tuned to 28 cycles with a band-width ratio of 2.5 This "delta filter" is very useful in dealing with irregular slow waves

Multiple "bipolar" recording This method employs two "active" electrodes on the cranium and no reference electrode Figure 1B shows the "four-points-in line" arrangement, which is one form of bipolar connection It will be observed that 2 amplifiers have an electrode in common For a description of the use of this type of recording for localization of tumors see Williams and Gibbs (1938) ⁴

"Triangle connections" shown in Fig 1C may or may not involve the reference point as one point in a closed triangle The principle is similar to that of the four-points-in line in that each amplifier records the activity from two points on the head By comparing the activity coming from each pair of electrodes, one may localize the source of specific or unusual activity (Jasper and Hawke, 1938) If the points are close to one another the amplification may have to be increased in order to bring out the detail Sometimes the activity under one electrode cancels out the activity under the other electrode (see also Kornmuller and Schaefer, 1938) It will be seen that this four-points in line differs from the triangle connection, not in principle, but by using the fourth point instead of returning to the first point in the triangle

Electrode placements In 1935-36 our electrode placements were on the mid line, in the occipital, vertex, and frontal areas The occipital placement is approximately 2 cm

⁴ Dr Goodwin of the Banting Institute in Toronto has pointed out (personal communication) that in bipolar recording an electrode over a tumor may act as a reference electrode The resultant record would be like a monopolar record because tumors are known to be electrically inactive

above theinion. The vertex is the point at which the frontal plane through the auditory meatus intersects the mid-line. The frontal placement is a point on the forehead in the mid-line just below the usual hair-line. More recently, with the 3-pen recorder, lateral placements 5 or 6 cm. on both sides of the mid-line have been added (cf. Fig. 1A) and for a more complete study a routine of 16-point placements divides the surface area of the head by spacing the electrodes at equal distances from one another, starting approximately 2 cm. on either side of the mid-line on the frontal, precentral, parietal and occipital areas. The resistances of the electrodes (solder pellets and Sanborn electrocardiogram paste) pasted with collodion on the scalp, are kept under 10,000 ohms as routine. High resistance may cut down the amplification and pick up extraneous artefacts.

Routine. With a tape-speed of 3 cm. per sec. and a sensitivity of 1 cm. for 100 μ V., a routine record is run for 20 min. as a minimum, up to 1 hr. as a maximum. First, several minutes of unfiltered record from one or more areas are taken. A filtered record is then taken at the increased amplification. During the unfiltered record and again during the 10-cycle record, the person is asked to open his eyes for 4 to 5 sec. and then close them again. This is done in order to bring out the modification of the alpha rhythm (Jasper, 1936). Next, an unfiltered record is always taken at increased amplification for detailed analysis.

Our most satisfactory routine for a 3-channel recorder is shown in Table 1.

If, from observation of the routine monopolar record, there appears to be anomalous electrical activity, multiple bipolar recording is employed to localize further the unusual activity coming from one or another part of the brain, which may necessitate the application of more electrodes to other areas.

MEASUREMENTS

Once individuals have been classified as normal, presumably normal, or abnormal and their EEG's obtained by standardized procedure, the problem is then to develop some method of evaluation which will differentiate between the EEG's of the normal and the abnormal group. A first glance at a collection of EEG's reveals in all records an interplay of waves and a fluctuation of voltages. Since many of the waves seen in the EEG's form groups of countable frequencies, we first selected frequencies for study and measurement. In 1935 and 1936 we made a detailed study of the EEG's of our first 50 subjects. At that time we had no filters and consequently counted frequencies wherever a half-second of time yielded at least 3 equally spaced waves, all of them at least 10 μ V. in amplitude. For each mid-line area 100 counts were made on sections of records where the eyes were closed, and we confirmed Berger's (1929) observation that the most common frequency was approximately 10 cycles. The frequency-distribution curve showed a blunt peak at 10, which fell off at $8\frac{1}{2}$ to 9 cycles on one side and at 11 to 12 cycles on the other side. The next peak in the distribution curve was much lower and was in a less well-defined range from about 14 to 20 cycles. This curve tapered off more gradually on both ends.

Alpha rhythm. We regard as "alpha rhythm" only those frequencies near

10 cycles which are characteristically suppressed when the eyes are opened and closed under standard conditions (Davis, H. and Davis, P. A., 1936). The momentary speed-up of the alpha rhythm by as much as 1 or 2 cycles following closing of the eyes is a characteristic modification pointed out by Jasper (1936). The *normal* alpha range is from approximately $8\frac{1}{2}$ to 12 cycles (which does not include the speed-up of an alpha following "eyes closed") and we believe represents the activity of a particular physiological mechanism especially susceptible to modification by internal conditions.

If the physiological activity of a normal person is depressed, as it appears to be when one becomes drowsy, the alpha activity may be altered accordingly. For instance, if one is very sleepy the alpha activity gradually disappears. If a subject is asked to open his eyes at this stage, the alpha activity reappears when he *opens* his eyes and disappears again when he closes them once more and can relapse to his former state. There are all gradations between this state and the alert state. The alpha waves may become irregular instead of reduced when eyes are open, but this is an indication of a state wherein the alertness is decreasing. The alpha activity increases when the eyes are closed in an intermediate optimum state which lies between the alert and the drowsy state. It represents a definite transitory level of physiological activity.

In our series of normal adult records, the alpha activity, modified as just stated, does not include frequencies below $8\frac{1}{2}$ or above 12 cycles. There have been a few exceptional cases in the "presumably normal" group with alpha frequencies of 13 cycles. In our series of records from *abnormal* individuals, there are a number with alpha activity including frequencies as low as, or even lower than, 8 cycles and as high as 14 cycles. In some of these records, frequencies of 16, 18 or even 22 cycles may follow closing of the eyes. This is not the accelerated alpha activity, but an abnormal response. With abnormal individuals, particularly those in whose EEG's dysrhythmic qualities are observed, the physiological mechanism responsible for the alpha activity is apparently influenced in a wide variety of ways. The alpha range is not clean cut and it is difficult to define clearly what the alpha activity really is in such a person. For instance, there may be a large amount of $7\frac{1}{2}$ to 8 cycle activity alone, or it may merge with 10-cycle activity, both responding like the alpha rhythm. If the frequencies are mixed, and the alpha frequency is to be defined, the speed-up on "eyes closed" should be carefully noted, in order to determine which is the alpha rhythm. Simultaneous recording through 3 filters tuned to 14, 10, and 7 cycles is invaluable for such a determination. If a record is completely 6 or 8 cycle (Fig. 5, sec. 2) and there is no 10-cycle activity, it *may* be characteristic for an abnormal person or for his condition at the time of recording, as, for instance, in certain conditions of impaired consciousness, or it may be a 10-cycle alpha slowed by drugs. What is significant is that the underlying physiological mechanism has been modified or is abnormal.

It will be noted that our use of the term "alpha" is interpreted from the

point of view of physiological mechanisms. It is these activities which we are attempting to measure. In other laboratories "alpha" is not so specifically defined (e.g., Harvey, 1939). Furthermore, it remains an interesting problem whether the 10-cycle frequency seen in rare instances in a certain stage of deep sleep, and also the 10-cycle or slightly slower frequency seen in the precentral area and not always clearly responsive to opening and closing of the eyes (Jasper and Andrews, 1938) are the same physiologically as the alpha rhythm seen in the occipital area. Until this problem has been worked out, some apparent contradiction will remain when workers in the field discuss the alpha rhythm, its behavior, and significance.

In all our studies of the EEG, the pattern of the occipital area in normal people appears to be the most stable, and, if an alpha frequency is seen in the EEG, the normal occipital record shows it more than any other area. As one moves from the occiput forward toward the frontal area the percentage of time the alpha frequency is present decreases (but cf. also Rubin, 1938).⁵ The parietal region is a transition area from occipital to temporal and motor regions. The temporal area is likely to show less alpha activity than the motor area. The EEG at the vertex is variable. The frontal EEG is often distorted by artefacts such as eye movements and muscle potentials. For these reasons, the occipital EEG was chosen as the basis for measurement of alpha activity. The definition of types of EEG pattern, to be described below, is also based primarily on the occipital record.

We do not agree with Jung's (1939) conclusion that in normal persons practically no alpha waves are generated in the anterior half of the brain. Jung explains the appearance of alpha waves in "monopolar" frontal and precentral records as due to changes of potential at the "reference" ear lead, induced by the activity of an alpha focus low in the occipital lobe. The characteristic equality in phase and voltage of frontal alpha waves in two hemispheres (in contrast to the independence of alpha activity in the two occipital lobes) lends some support to Jung's suggestion, which is a theoretical possibility and must not be overlooked. Nevertheless, the explanation of *all* frontal and precentral alpha activity by Jung's hypothesis requires complicated assumptions as to shifting foci of alpha activity which seem to us implausible. A more detailed discussion is beyond the scope of this paper, as the problem of *source* of alpha or any other type of wave in no way affects the empirical classification, evaluation and interpretation of EEG patterns, which is our present concern.

Alpha index. The alpha index is the percentage of time the alpha frequency appears in the monopolar occipital record, at a level 2 cm. above theinion (Davis, H. and Davis, P. A., 1936). The broadly tuned 10-cycle filter greatly facilitates the measurement. At least one (preferably more) standard length of 100 cm. of record run at 3 cm. per sec. and taken under standard conditions at least 10 sec. after the eyes have been closed is chosen. The alpha index is the number of centimeters occupied by the alpha rhythm in such a sample. The frequency of the alpha rhythm of an individual rarely varies more than 2 cycles. If his alpha frequency is 10 per sec. it may speed up 1 or at most 2 cycles following "eyes closed." Often it helps to get the alpha range by counting the first train of waves (average frequency of the

⁵ Rubin does not specify precisely the frequencies which he calls the alpha rhythm.

first second) following "eyes closed" if they come in within the first half-second. This is the upper limit. Two cycles below this upper limit defines the lower limit. We arbitrarily include only those alpha waves appearing in trains of at least three waves, all of them 7 μ V. or more from peak to trough.

The alpha index was the first objective measurement of the EEG by which subjects were divided into groups. We divided our first 50 subjects into 4 groups—the "dominant" being those with an alpha index from 75 to 100 per cent; "subdominant" from 50 to 75 per cent; "mixed" from 25 to 50 per cent; and "rare" from 0 to 25 per cent (see Davis, H. and Davis, P. A., 1936). The original 50 were almost evenly distributed, due to the small number of individuals, but our more recent measurements on a series of 400 "presumably normal" and "average" individuals (cf. p. 97) show a distribution with a definite peak at about 70 to 80 per cent (Davis, H., 1938; Davis, P. A. and Davis, H., 1939) and also confirm the approximate constancy of the index for a given individual over a period of years.

The alpha index was also measured on an "abnormal" series of 100 mental hospital patients (Davis and Davis, 1936). The peak in the distribution curve for this abnormal group was toward the lower indices, but measurement of this single factor alone did not differentiate an abnormal individual from a normal person.

Walter (1936) suggested "delta" as a generic term for slow waves. We use the term in a somewhat more restricted sense to include irregular wave-lengths greater than 2.5 sec. or frequencies up to and including 4 per sec. (Davis, H., 1938).

Delta index. Hoagland introduced another measure which he called the "disintegration factor" (Hoagland, Rubin and Cameron, 1936) or "delta index" (Hoagland, Cameron and Rubin, 1937). Regarding the slow-wave activity (slower than alpha waves) seen in the EEG as a tortuous line, he traced its length in 100 cm. of record by means of a map measurer. The excess length of the line above 100 cm. he then designated as the delta index. He wished to measure the amount of slow-wave activity because of an impression that the frequency range below the alpha indicates abnormal physiological function. He applied his method to the records of schizophrenic patients in an attempt to find out whether the index correlated with the clinical status of the patient, but found no correlation between the index and schizophrenia. We measured the delta indices of our mental hospital group and compared the slow-wave activity with that of normals. The delta index was not a measure which would indicate the degree of clinical abnormality in a person, nor would it select the schizophrenic individuals from a mixed group.

The real difficulty with Hoagland's delta index, we feel, is that he attempted to measure abnormality which is represented both by irregular *voltage* and by long *wave-lengths*. These are two independent functions which do not bear the simple relationship to one another that the single figure of

the delta index implies. The abnormalities defy mathematical simplification. In the same length of record, a person having a large amount of very slow 1- and 2-cycle wave activity would have a lower delta index than another person having a lesser amount of 3- and 4-cycle activity of equal voltage. Yet the degree of abnormality represented by the 1- and 2-cycle range is often greater than that indicated by faster waves. We attempted to improve Hoagland's delta index by confining the measurement to a narrower range below 5 cycles, but still failed to find the measure valid. We called the measure so obtained the "delta excess" (Davis and Sulzbach, 1940).

Wellenindex. Jung (1939) also found the delta index unsatisfactory and devised instead his "Wellenindex," or wave index, which is the product of the greatest amplitudes and longest wave-lengths in a sample of record. Measurements are made in units of 100 μ V. and 100 msec., and only such maximal amplitudes are considered as appear at least three times in 10 sec. The 3 maximal values are averaged if necessary. In healthy people, according to Jung, the wave index is generally less than 1, never above 1.5; in epileptics mostly above 1 up to 6, and in petit mal up to 30. In cases of brain tumor the wave index is less useful.

Measurement of coördination. Jung (1939) also undertook to measure the degree of coördination of different brain regions, particularly of the two occipital lobes with respect to alpha activity. Such measurement involved a statistical analysis of the phase relationships existing from time to time between the two sides of the head. No simple single "measure" or index has yet emerged from this study, but merely the statement that most "normals" show an "in-phase" relationship from 80 to 90 per cent of the time.

Grass-Gibbs frequency analyzer. Grass and Gibbs (1938) have developed a precise method for measuring the energy distribution as a function of frequency, but it is too early to judge its practical value. They have applied the method to the EEG's of many normal and abnormal individuals under varying conditions, *i.e.*, epilepsy, shifts in acid-base balance, sleep, etc. (Gibbs, Williams, and Gibbs, 1940). If amplification and sensitivity of the amplifiers are controlled, an accurate graphic representation may be made automatically of the distribution of energy throughout the spectrum in a given period of time (usually 30-sec. periods) chosen from the total record. This method is far more precise and objective than any method of subjective judgment based on visual inspection, even with the aid of broadly tuned filters. An important limitation, however, is that the analysis does not reveal the phase-relationships (wave-form) of the waves and their sequences in time. The method of analysis averages 30 sec. of activity and does not give any information as to whether the energy is produced by an even distribution throughout the 30 sec. or by a brief episode of extremely high voltage. This is important and will be discussed later. The frequency analyzer gives a separated series of static values to a dynamic progression of activity, but the method may well prove to be a valuable tool, particularly when selected features within sections of an EEG are to be studied.

TYPES OF ELECTRICAL ACTIVITY

The accumulation of experience has led us to approach the problem of evaluation from still another angle, namely, observing the records in detail as a complex of inseparable and variable relationships. Frequencies and their relationships to one another, as well as voltage fluctuations, are studied. We now regard the complicated electrical activity as a *pattern* of electri-

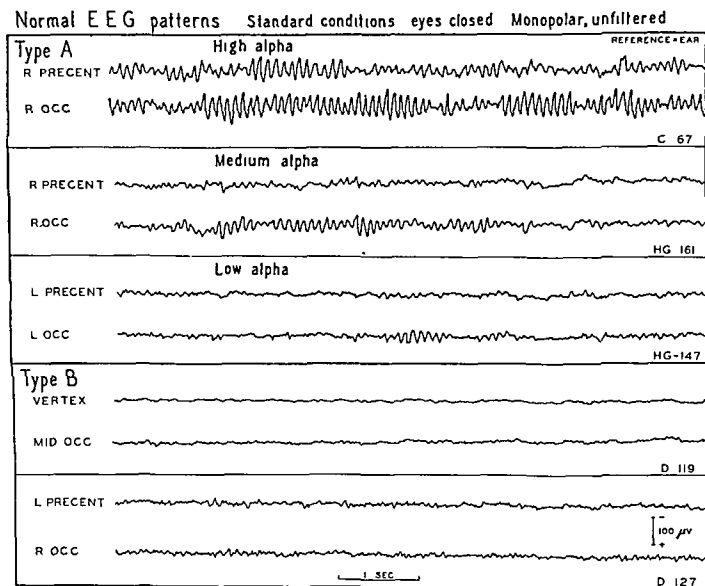


FIG 2 In this and all subsequent figures, upward deflection indicates that electrode on head is electrically negative relative to reference electrode

cal behavior rather than as a series of separate factors, in the same manner as one regards a person's behavior, attitude or personality as a pattern and not a series of separate factors.

Beta activity. The beta activity is a universal feature of all records and is present all over the living brain. It consists of short wave-lengths and fast frequencies (above 20 cycles). It represents physiological activity of the brain and is modified by physiological changes such as sleep. It is generalized, formless, low-voltage activity and serves as a rough background from which all patterns emerge. One can appreciate the beta activity of the brain

by observing the "residual occipital activity" when the eyes are open in a bright light. Under these conditions the alpha pattern is suppressed and makes clearer the beta activity. With experience it is possible to distinguish the beta activity from muscle potentials and from the "choppy" type of activity to be described below.

Patterns of electrical activity in normal individuals. Every individual when recorded under standard conditions reveals a pattern of electrical activity which is characteristic for him at his resting level. It must be emphasized that we are dealing with a dynamic physiological equilibrium and not a

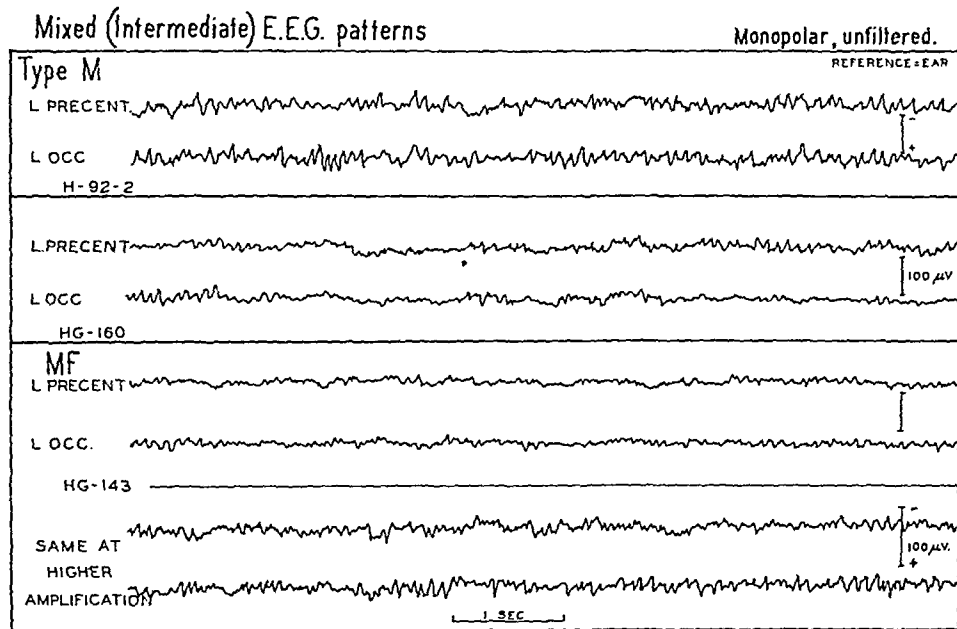


FIG. 3.

static condition. He may have an EEG of a high-voltage, predominantly alpha pattern or he may have a low-voltage pattern in which fast frequencies dominate. Whatever picture his EEG pattern presents, it maintains the same characteristics of wave-form, frequency range and voltage fluctuation upon repeated recording from one year to another, *provided* he is recorded under the same standard conditions.

A is the type of pattern (Fig. 2) which has a regular and clearly countable alpha rhythm which reveals a proportional distribution (cf. p. 101) over the occipital, precentral, and frontal areas when recorded *simultaneously*. Other frequencies in the pattern are consistent in their distribution and do not destroy the regularity of the alpha trains. The pattern may have a low alpha index and low voltage, for it is *quality* rather than *quantity* which is impor-

tant The average voltage fluctuation of a typical *A* type of pattern is from 30 to 80 μV Most *A* patterns have a characteristic voltage of 50 μV or higher

B is the type of pattern (Fig 2) which under standard conditions and resting level of activity is made up predominantly of fast frequencies from 14 to 20 cycles which merge into the background of low-voltage beta activity The average voltage fluctuation of a *B* type of EEG is from 10 to 30 μV , rarely higher This type is rare but definite

M is a type of pattern (Fig 3) which is composed of mixed frequencies, none clearly dominant, but which includes such a wide frequency range that

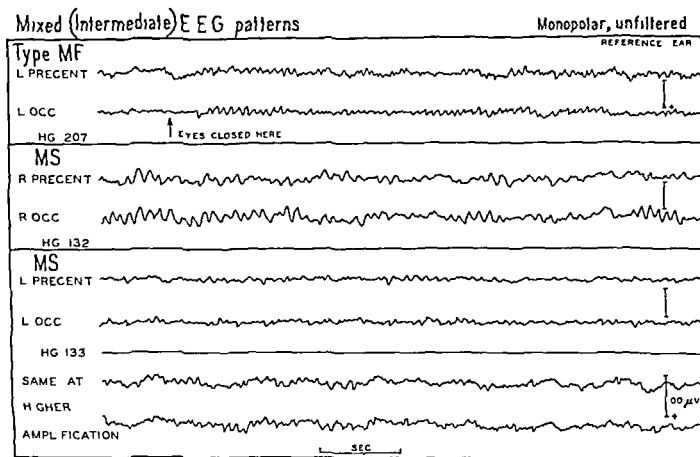


FIG 4

slow, alpha, and fast frequencies are all represented The range of voltage is usually between 20 and 60 μV The irregularity of the patterns in this group is in contrast to the uniformity and regularity of the *A* and *B* patterns

MF indicates a mixture of frequencies (Fig 3 and 4) in the alpha and fast frequency ranges whose voltage fluctuates in a consistent manner within a narrow range These patterns show varying amounts of alpha activity mixed with a fast frequency component which tends to make the alpha wave form sharp or irregular The frequency of the alpha is usually over 10.5 cycles The *MF* type of pattern is the commonest among normal individuals, although the *A* type is a close second

MS is the counterpart of *MF*, and includes EEG's having a mixture of frequencies (Fig 4) in the alpha and the slow-frequency ranges These alpha

waves are likely to be in the 9- to 10-cycle range and slightly blunted or deformed by the interference of the slower frequencies.

If there is difficulty in making a decision between the *A* and the *MF* type, determining factors for *MF* are the departure from the proportional distribution and from the regular quality of the alpha activity over the various areas, and a greater prominence of fast or slow activity in the precentral and frontal regions.

These groups, *A*, *B*, *M*, *MF* and *MS*, are regarded as normal when they maintain a consistent stability of voltage and frequency range for their type. The divisions between these groups are arbitrary and often difficult to determine, because the quality of variability by its very essence precludes a rigid classification.

GENERAL TYPES OF ABNORMALITY SEEN IN THE ELECTROENCEPHALOGRAM

As one studies EEG patterns over a long period of time, it becomes increasingly clear that as a pattern becomes less organized it becomes less normal (Fig. 7). This statement is based on many observations in which the

Abnormal E.E.G. Episodes

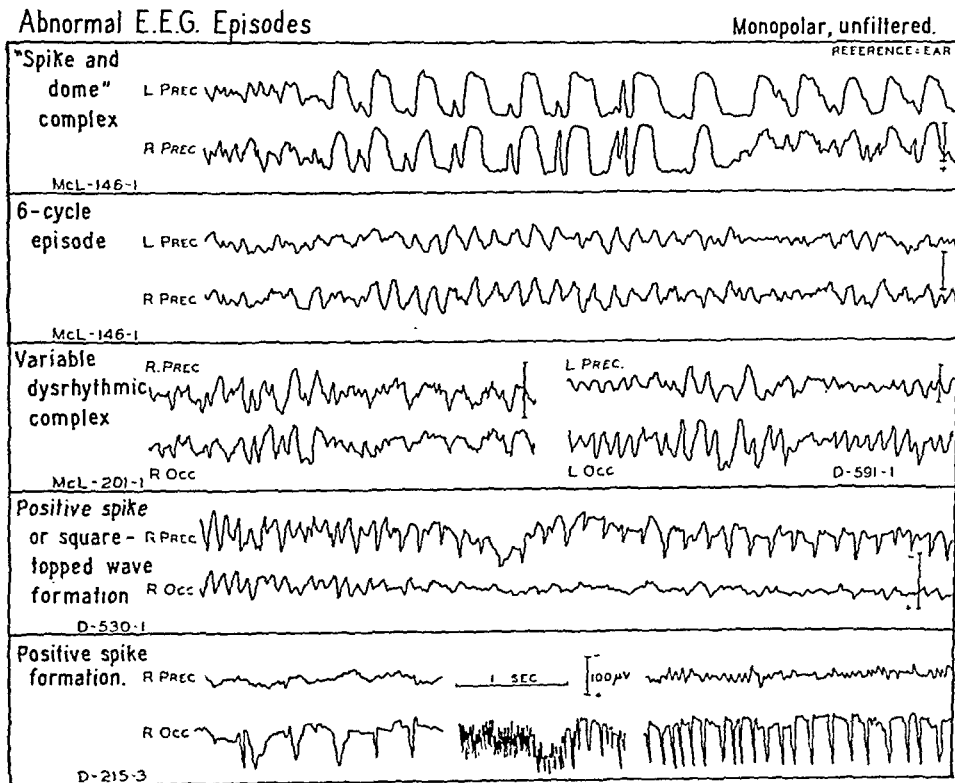


FIG. 5. Types of abnormal activity seen among mental patients. These examples represent transient abnormal physiological behavior of the brain.

clinical condition of the individual has been correlated with the modifications of the EEG. As the instability becomes exaggerated, the pattern begins to break up into episodes (Fig. 5) which involve a remarkable assortment of configurations made up of extremes of frequency, voltage, or exaggerations of one or few frequencies, usually outside the alpha range.

The type of disturbance in one or more regions of the brain determines the significance of the abnormality.

"Choppy" type of activity. This is an abnormal, formless pattern which looks like beta activity at increased amplification on a slower tape (Davis,

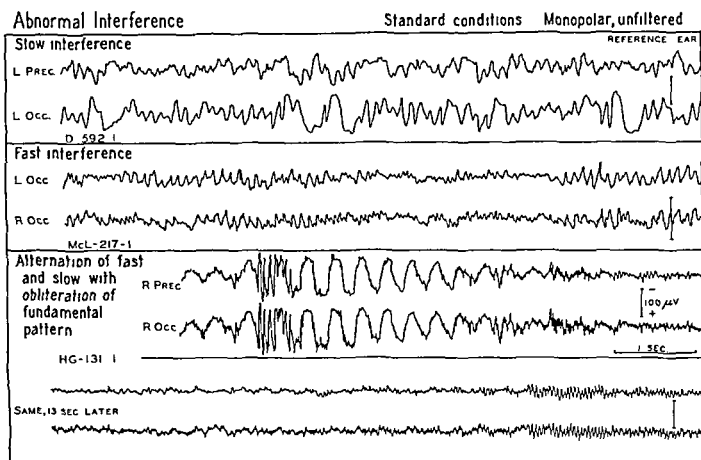


FIG. 6.

P. A., 1940). For two years this quality in the record was confused with muscle potentials. We felt that it was not due to muscle potentials, because after using every means at our disposal to rule them out the pattern still remained in certain cases. Finally, with hospital patients upon whom pneumoencephalograms or x-ray plates were made, it was possible to show that this "choppy" activity was associated with regions of gross cerebral lesion. If it were muscle potentials, other leads in corresponding areas on the opposite side of the head should have at least occasionally revealed the same type of record. One record on an individual may not be enough to determine whether the "choppy" quality represents muscle potentials or whether it is due to cerebral lesion. One must use bipolar as well as monopolar recording, compare corresponding areas of the head and use every precaution to rule

out artefact or muscle potentials. The "choppy" quality has been seen only very rarely outside the hospital, and at present we feel that it indicates cerebral damage.

Dysrhythmic type of activity. The dysrhythmic type of abnormal interference includes the *well-known abnormal complexes* (Fig. 5 and 6) which may appear and disappear in the EEG. It may be well organized (Fig. 6, HG-131) or unorganized (Fig. 6, D-592; Fig. 7), or it may appear as a variant which never establishes itself clearly as a well-organized interference (Fig. 5, secs. 3, 4). "Dysrhythmia" implies disturbance of the normal rhythms of the brain; it does not imply an arrhythmia or necessarily an irregularity in trains of waves. It is used here in the sense that Gibbs, Gibbs and Lennox

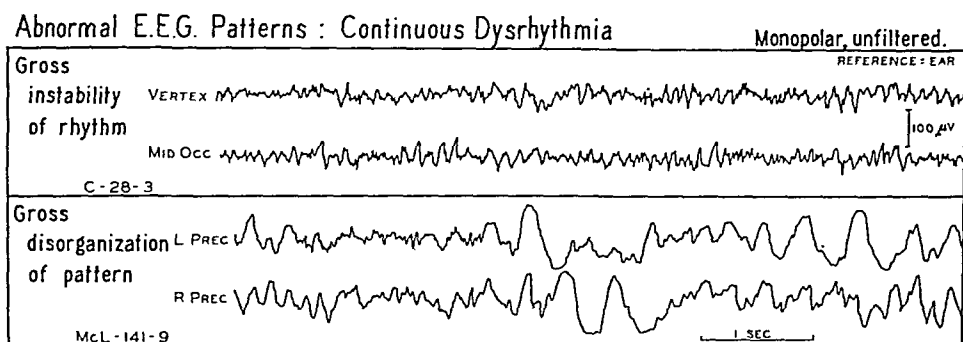


FIG. 7. Two examples of continuous dysrhythmia. The first represents gross instability which interferes with but does not destroy the fundamental pattern. Electrical filters introduced into the recording circuit would reveal more clearly the fundamental alpha pattern of this EEG.

The second EEG represents gross disorganization, in which the alpha activity is replaced by the delta activity which destroys the fundamental pattern.

(1937) employ it in describing the unusual patterns recorded in "psychomotor" attacks and other epileptic seizures.

The dysrhythmic types of abnormalities may be listed as follows:

(1) *Localized slow-wave activity* is observed in the region of tumors or other gross lesions.

(2) *Episodes of "locked patterns"*⁶ include the "spike-and-dome" complex, often associated with petit-mal, the minor seizures of epilepsy with brief lapse of consciousness (Fig. 5, first 2 lines), and the organized trains of waves of fast frequency and of high voltage, usually characteristic for grand-mal or overt convulsive seizures. The less clearly organized episodes of fast, positive-spike, or square-topped waves (Fig. 5, secs. 3, 4, 5) are observed in all types of epileptic seizures, but are perhaps more commonly seen in relation to attacks of confusion, fugues, behavior disorders and atypical motor attacks (types of seizures all combined under the title of "psychomotor" by

⁶ "Locked pattern" is a term defining a dysrhythmic combination of wave complexes which follow each other in organized sequence.

Gibbs and Lennox). These EEG patterns are not as well organized as the "locked patterns." In fact, any specific frequency (Fig. 5 and 6) interfering with the characteristic normal pattern may constitute an episode.

Such abnormal activity indicates a temporary disturbance of physiological function. Correlation of some of these formations with behavior or symptoms is so striking that their appearance in less organized form, without clinical symptoms, is important.

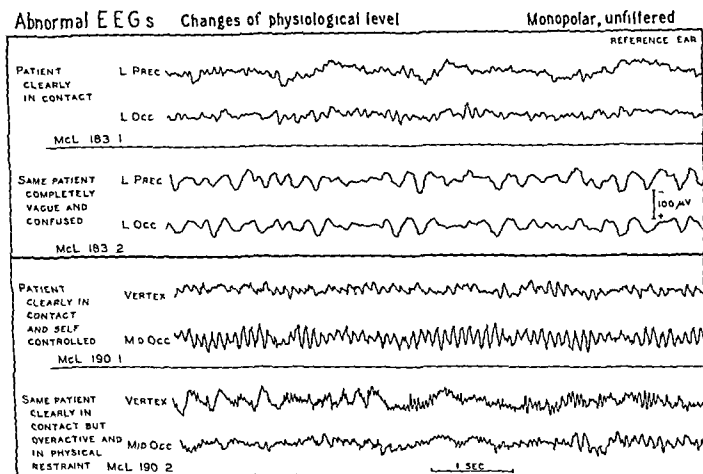


FIG. 8 Abnormal EEG's of 2 mental patients. McL-183 shows delta activity which is associated with impaired consciousness at the time of recording "In contact" implies that the patient is aware of and responsive to his immediate environment.

McL-190 shows fast-frequency activity replacing the alpha and maintaining itself in the presence of delta waves. This electrical activity is associated with clinical overactivity at the time of recording.

(3) *Continuous generalized dysrhythmia* may continually distort the EEG to such an extent that the characteristic pattern of the person is *never free from the distortion* (Fig. 7 and Fig. 6, D-592). The transitions from the normal EEG to the abnormal EEG are many. *Episodes* are periods in which there is a transition sharp enough to stand out clearly from the characteristic pattern (Fig. 5), or a gradual build-up into a frequency range of voltage change sufficient to differentiate or delimit the activity from the rest of the record (Fig. 6). Episodes may make the characteristic pattern abnormal only temporarily. On the other hand, an inherent disorder in the mechanisms

which modifies the activity of the cortex may continuously interfere with the establishment of a normal pattern (Fig. 7).

It is known that under 10 years of age children's patterns normally tend to have slower frequencies and are more unstable than those of adults. Therefore, if one is to understand the significance of variations in patterns, it is important to know at least the approximate age of the person when evaluating an EEG, as well as to have had experience with different types of EEG.

An EEG is a record of continuous activity fluctuating within certain narrow limits of frequency and voltage. When, under standard conditions of recording, the EEG fluctuates beyond a certain point and the frequencies reveal a spreading or clumping in some unusual manner it is an indication of greater instability of physiological activity (Fig. 5, sec. 3, 4, and 5; Fig. 7, sec. 1). From rambling low-voltage abnormal activity, creeping into and disappearing from a record, to the recognized specific complexes which are organized as "locked patterns" (Fig. 5, sec. 1, 2, 4, 5), one may observe every gradation in quality, relationship, and degree (Fig. 7 and 8).

EVALUATION OF THE TOTAL RECORD

In the Department of Physiology at the Harvard Medical School and the electroencephalographic laboratory of the McLean Hospital we have been studying the mechanisms underlying these abnormal complexes which break into the individual characteristic pattern. Disturbances in the fundamental mechanisms of the brain sometimes completely obliterate the pattern, as in the epilepsies, or only modify it locally, as in focal cerebral lesions. Experience and detailed study of the conditions under which they appear or can be produced must be the basis of evaluation.

Certain alterations of electrical pattern are correlated with particular types of alteration of behavior. The alteration of behavior may be over-activity (see Fig. 8, last 2 lines), or a greater retardation of activity (Fig. 8, first half). Slow and fast frequencies, which may occur singly or combine to form a complex or particular pattern of their own, are often associated with overt behavior disturbances. Many epileptic patterns, especially the "spike-and-dome" complex, represent a combination of fast and slow waves (Fig. 5, sec. 1, 3, 4, 5). The important factor is the *change of electrical activity* from what is normal for the individual. The *changes in quality* of electrical activity constitute a basis of evaluation.

A gross analysis of the EEG recorded by routine placements has been standardized (Table 2).

Rating the record on a five-point scale. The basis of ratings is not specifically frequency or voltage. It is based on the quality of the pattern as a whole, particularly as to its general stability, regularity of wave-forms, and the type and amount of variability or interference if any.

Rating 1 is given to any normal type of pattern which is stable in its fluctuations of frequency and voltage within fairly narrow limits and without

any sharp transitions. The pattern of each area is consistent throughout the record and there is an approximate similarity of patterns and voltages in the two hemispheres. The patterns in the different areas bear the usual relationships to one another (see p. 108).

Table 2 *Tabular headings for gross analysis*

Serial no	Type of pattern	Volt	Reg	Alpha		Resp to "O & Cl"	Asymmetry	
				Per cent	Freq		Yes (+) No (-)	More α on right or left
	A, B, M, MS, or MF	Ave (+) High (++) or Low (-)	Ir, Int, or Reg			Good (+) Poor (-) or Ir		

Abnormal activity					Freq band of Dysrhythmia			Location of Dysrhythmia	Rating	Remarks
E	R	Sp	Diff	Local	δ 1-3	SF 4-8	FF 16+	If not diff give the order of prom by area	Based on total record	

Key

Ir =irregular, meaning frequencies are irregular and distorted

Int =interference, meaning good wave form but other frequencies interfere with its appearance or superimpose themselves

Reg =clearly regular without distortion or superimposed frequencies

Alpha per cent is measured on 100 cm of record

Alpha frequency is counted and determined from speed-up following eyes closed (see text)

Resp to "O and Cl" is appearance of alpha following eyes open and closed Slight delay in response is secondary

Sp =specifically recognized complex such as petit-mal or grand-mal complex complex or variant

Diff =diffuse

Local =localized to an area or side

E =episodal dysrhythmic interference

R = "rambling" dysrhythmic quality as opposed to clear episode, usually in the slow range

Rating 2 includes normal records which are slightly less stable or regular than those rated 1, and which may fluctuate in a somewhat atypical manner or which have an alpha rhythm that is regular but unusual in wave-form. If in an otherwise normal record there are *atypical* eye-movement patterns, which may or may not be due to movement artefact, when the eyes are either open or closed, it rates as 2.

Rating 3 includes normal records in which some feature may be exaggerated and yet which cannot in itself be regarded as abnormal The *M* group of patterns are often rated as 3 because of the quality of instability, alteration of frequency and distortion of wave-form. Rating 3 is also given to unusual records which are atypical but about which nothing is known defi-

nitely enough to classify them as abnormal. A record rated as 3 is different from normal records, yet so free from any specific known abnormality that it is placed on the borderline until further knowledge gives us a clue as to how it should be classified. A pattern in which more alpha frequency appears on the frontal area than on the occipital area,—everything else being equal,—would rate as 3. The record as a whole and the relationships of the activities in all recorded areas must be regarded.

Rating 4 is given to any record which is dysrhythmic and suspicious and has recognized abnormal qualities clearly indicated, but no feature typical or

A comparison of the distributions of the E.E.G. ratings of 681 individuals.

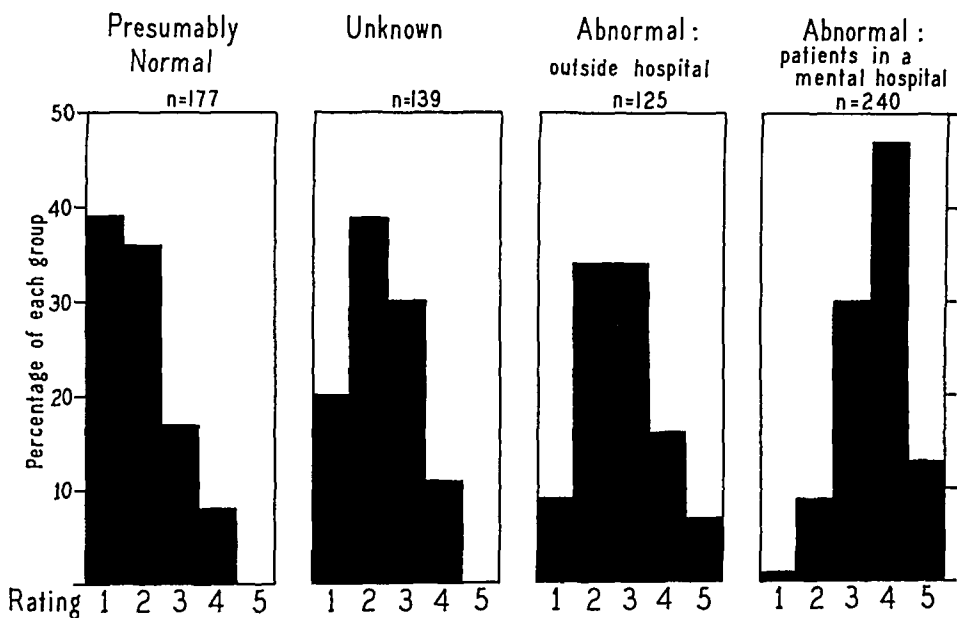


FIG. 9.

outstanding enough to warrant definite diagnosis. Included in a group rated as 4 would be many patterns which have irregularities maintained for very short times or contain waves or formations which are outside normal limits but not sufficiently organized to be characteristic of definitely abnormal episodes. The irregular and unsteady quality of such a record becomes in itself the dominant characteristic rather than any one particular feature or the sum of a group of features.

Rating 5 is given to any record which reveals recognized abnormal dysrhythmias, such as those found in epilepsy (Gibbs, Gibbs, and Lennox, 1938a, b). The abnormalities must be specific and well known. "Choppy" records, especially if obtained from all leads and controlled by tests to rule out artefacts, would be rated as 5. If slow waves can be localized such as those suggestive of a tumor or other gross lesion, the record would be rated as 5.

These ratings involve a consideration not only of the type of abnormality seen in the EEG but particularly the factor of stability, which is an essential characteristic of ratings 1 and 2, and the factor of instability characteristic of ratings 4 and 5.

Even within a group of known abnormals such as a group of schizophrenics (Davis, P. A., 1940) it was shown that the factor of stability and instability correlated with clinical behavior. Normal stable patterns were correlated with stable behavior, although stable behavior does not here imply normal behavior. Dysrhythmic patterns were correlated with unstable behavior.

Validation of the rating system. The EEG's of the groups of normals, presumably normals, unknowns and abnormals were rated, the groups being mixed and the EEG's given numbers so that the identity of the individuals was unknown. The author re-rated 121 EEG's and agreed with her previous ratings to within 1 point in all but 7 cases. A second judge, Dr. H. Davis, agreed to within 1 point with the author in all but 21 of 134 cases, and within 2 points in all but 2 cases.⁷ There was complete agreement on the cases falling in the extremes when all EEG's containing episodes which might be artefact were eliminated. In no case was there a shift in the rating from normal to abnormal, or *vice versa*.

Only 14 individuals met all the requirements of the classification of "selected normal." It is significant, however, that both judges independently and without any knowledge as to their identity or classification gave their records all a rating of 1. This group was so small they were added to the "presumably normal" group.

The data validate the system of measurements presented in this paper. The peak of the distribution curves (Fig. 9) shifts systematically from 1 to 4 as we go from the normal to the abnormal group. No EEG's in the groups of normal and unknowns are rated as 5, which is the rating given to an EEG which we consider diagnostically abnormal. There are a few, but only a few, EEG's rated as 1 in the abnormal groups. The correspondence between our ratings of the EEG's and the groupings of the individuals validates the criteria of normality and abnormality of the EEG.

The graphs show an overlap in the ratings. Further study may reveal the reasons and significance of this overlap. Hereditary factors in epilepsy (Löwenback, 1939; Lennox, Gibbs and Gibbs, 1939; Strauss, Rahm and Barrera, 1939) offer an important lead in this direction. It explains the rating of 4 in a number of individuals considered clinically normal, for some of them have epileptic parents or blood relatives. These normal people carried the same abnormal qualities in their EEG's which are seen between seizures in the EEG's of known epileptics.

DISCUSSION

There is some overlap of the A, B and M types of pattern. The extremes, however, are clear. The A type of pattern is different from the B and M

⁷ The ratings were first made on a 9-point scale as follows: 1, 1a, 2, 2a, 3, 3a, 4, 4a, 5. A 1-point difference exists when ratings 2 and 3, or 2a, and 3a, appear for the same record, but 2 to 2a, or 2a to 3 are only half-point differences.

types in spite of the fact that the brains revealing them are apparently normal. Human beings are equipped with similar physiological mechanisms and gross anatomical structure, but this does not imply that the brains will respond in similar manner.

The EEG reflects the behavior of the brain as it maintains its level of activity in its internal environment. The internal environment is kept rela-

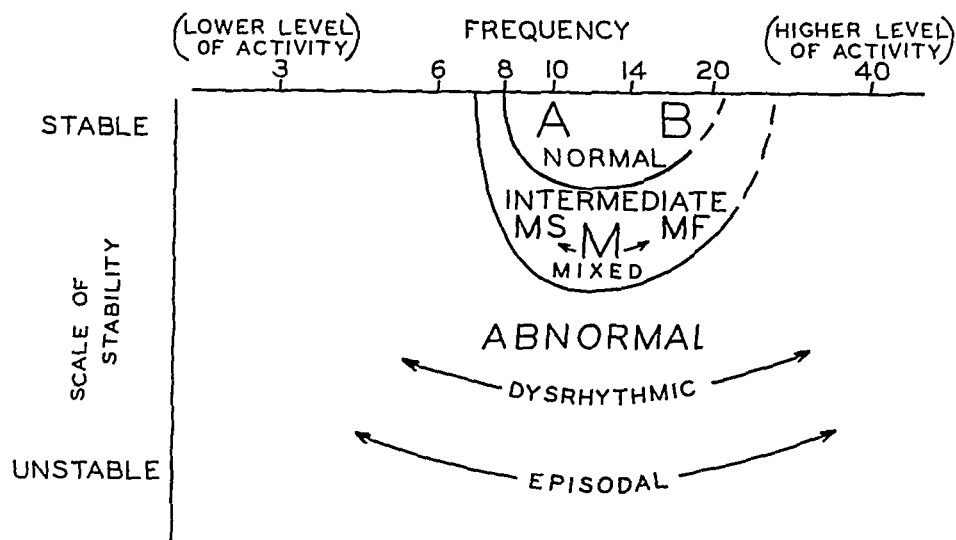


FIG. 10. Schema of the physiological activity of the brain as expressed by EEG patterns in terms of normality, stability, and frequency.

Although most frequencies are represented in all EEG's, certain ranges stand out most clearly in a particular record, and place the type of pattern in this schema. The gradation from normal to abnormal involves a wider spread of the frequency range and at the same time a tendency to greater instability. When a record is more confused with slow frequencies there is usually a distinct shift toward disorganization and breakdown of frequencies into irregular waves or complex formations (lower left quadrant). When the higher frequencies are involved there usually is a tendency toward greater organization and regularity (upper right quadrant). Correlated with these extremes, there is at the slow end impairment of consciousness from a depressed physiological activity and at the fast end physiological overactivity which may eventually lead to exhaustion with resulting impairment of consciousness.

This schema includes only two dimensions of a multidimensional schema which is in the process of development.

tively constant by the familiar mechanisms of homeostasis. If the internal environment of the brain is modified, the EEG will reflect the change, but brains have different resting levels of activity. It should be stated here that these levels of activity do not appear in any way to be related to intelligence, as the word is commonly used.

Figure 10 represents certain aspects of the physiological activity of the brain, as expressed by the various types of EEG patterns, in terms of stability, frequency and normality. It is intended to express graphically our generalizations that the unstable dysrhythmic or episodic records are abnor-

mal and also that a record whose most prominent frequencies are very fast or very slow is likewise abnormal, even though the pattern may be well organized and stable.

If the anatomical structure of the brain is damaged or its physiological or chemical condition is modified to the extent of altering the state of consciousness the EEG will be modified (Davis, H. and Davis, P. A., 1939). The general types of abnormal electrical activity have been described in order that the significance of disturbances of physiological function may be appreciated when evaluations of the EEG's are being made. Moreover, a relation between a person's EEG and his characteristic behavior has been found (Davis, P. A., 1940). Types of normal EEG's and their modifications are being related to subtle changes in behavior of the individual as well as to the more fundamental psychological organization of the personality (Saul, Davis and Davis, 1937; Davis, H. and Davis, P. A., 1939).

SUMMARY

The measurement of specific features of the EEG is described. The limitations in measuring single factors, and of synthesizing them, is pointed out. A system of evaluating the EEG pattern as a whole on a 5-point scale from normality to abnormality is described (p. 108). The underlying quality of stability versus instability of pattern is the basis of this system of evaluation.

Normal patterns are divided into types as follows: *A* patterns (Fig. 2) are dominated by regular alpha sequences which are clearly countable. *B* patterns (Fig. 2) lack alpha sequences when subjects are run under standard conditions. *M* patterns (Fig. 3) include EEG's which are composed of mixed frequencies. This latter group is subdivided into the *MS* group (Fig. 4), in which the slow waves are responsible for the mixed frequencies, and the *MF* group (Figs. 3 and 4), in which the fast frequencies are prominent. The *M* group, though regarded as normal, appears to be intermediate between the normal and abnormal.

The abnormal features of the EEG known at present are described as "episodal" (Fig. 5) or as "continuously dysrhythmic" (Fig. 7). They include every gradation of dysrhythmia up to the well-recognized epileptic patterns; the "choppy" quality obscuring a normal pattern (shown to have been associated with brain damage in three cases); and localized slow-wave activity associated with tumors or focal lesions. These features are discussed in relation to the evaluation of an EEG (p. 105).

The importance of the technique and procedure of recording and the necessity for standard conditions are emphasized.

The method has been validated on selected groups of adult individuals which are compared with one another, such as a known normal group, a presumably normal or average group, and known abnormal groups (Fig. 9).

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ACTIVITY IN NEURONS OF THE BULBOSPINAL CORRELATION SYSTEM*

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THIS STUDY is concerned with the descending spinal projections of bulbar and spinal nuclei. These find their anatomical expression chiefly in the vestibulospinal, reticulospinal and propriospinal tracts. It is certain from anatomical studies that stimulating electrodes placed in the floor of the fourth ventricle excite neurons of the vestibular and reticular systems (Gray, 1926, Papez, 1926, Lorente de N6, 1938c). A diagram of vestibuloreticular connections and their ascending projections has been presented by Lorente de N6 and Berens (1939, Fig. 4). Stimulating electrodes, placed within the ventrolateral columns of the spinal cord, excite not only the spinal projections of the vestibuloreticular complex, but also neurons of the propriospinal system which arise at, or cephalad to, the site of the electrodes (Sherrington and Laslett, 1902, 1903†).

The results of stimulating these bundles have been recorded by the use of microelectrodes placed either in the tracts themselves, or in the gray substance, and by leads placed either on ventral roots, or on the peripheral plexuses, to record the discharge of motoneurons. The first attempt to obtain records of the activity of the spinal cord and associated nerves following stimulation of the cord itself, was made by Gotch and Horsley (1891). Working under many handicaps that have been remedied in the intervening 50 years, these investigators observed only the slow potentials which may be recorded from the surface of the cord as a result of tetanic stimulation. Some of their original observations have been repeated during the course of the present investigation. However, the observations reported here deal with the more rapid and detailed responses observable with single shock excitation, which unavoidably escaped the attention of Gotch and Horsley.

Cats have been used throughout the experiments. The preparations have been decerebrate, decerebrate spinal, or spinal, or lightly narcotized with dial (usually 0.5 ml/kg) or nembutal (25–30 mg/kg) with or without subsequent spinal section or decerebration. The observed differences in these preparations were largely quantitative, and were probably related to the intensity of tonic vestibular background.

* A preliminary account of some of the present results was presented at the meeting in New Orleans of the American Physiological Society (Lloyd, 1940).

† Extracts from this classical paper are reprinted in *Selected writings of Sir Charles Sherrington*, compiled and edited by D. Denny Brown, New York, Paul B. Hoeber, 1939, xiv, 531 pp. (see pp. 201–224).

Conduction in bulbospinal and propriospinal tracts

The records reproduced in Fig. 1 show the response of the ventrolateral columns to a single shock delivered to the floor of the fourth ventricle at each of several conduction distances varying between 3 and 29 cm. The characteristic response consists of two spike potentials separated by an interval of 0.9 msec. In other preparations this interval has varied from 0.7 to 1.0 msec. Although the latency to the first spike potential increases with the conduction distance, neither of the two spike potentials suffers appreciable temporal dispersion, nor is the interval between the spike potentials increased, even after conduction through the whole length of the spinal cord. These facts are consistent only with the interpretation that conduction is occurring in two groups of homogeneous fibers of alpha velocity, separated in time by an interval characteristic of a single central synaptic delay (Lorente de N6, 1935a; 1938a).

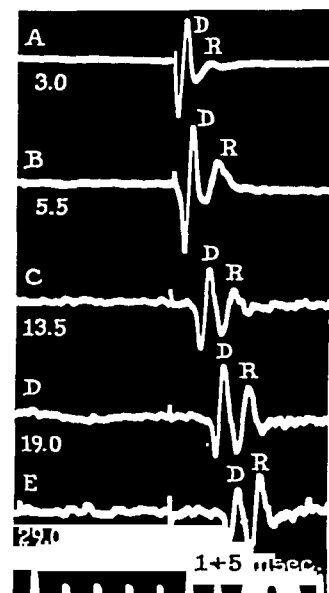


FIG. 1. Bulbospinal preparation. Activity in the ventrolateral column recorded at various levels of the spinal cord by a microelectrode placed in the column. Stimulus, a single shock to the floor of the fourth ventricle. Conduction distance in centimeters is indicated for each record by the figure at the left. D, signifies the directly conducted alpha spike potential; R, the synaptically relayed alpha spike potential. Time in 1 and 5 msec. intervals is shown at the bottom. In all the figures where there are two time designations, these are for the small and large divisions respectively.

Further evidence that the second spike potential of the column response is recorded from neurons excited transsynaptically is found in Fig. 2. In this experiment the stimulating electrodes were placed in the lower thoracic cord in a way so as to stimulate fibers of the ventrolateral columns. The recording microelectrode was placed in the column of the lumbar cord 9 cm. aborally. Two shocks, separated by an interval of 2.5 msec., were delivered. In Fig. 2A, the characteristic double spike potential response which follows each of the two shocks is seen. The second or relayed spike potential of the response to the second shock is increased by facilitation. Other examples of the facilitation of the relayed spike potential may be seen in Fig. 7 and 8. The relayed spike potential is rapidly abolished by a period of asphyxia too short to decrease or disperse the initial spike potential appreciably. Figure 2 (B and C), otherwise identical with Fig. 2A, shows two stages in the effect

of asphyxia on conduction through the ventrolateral column. A completely differential and rapid loss of excitability such as this is characteristic of central synapses, rather than of different fiber components directly stimulated but suffering dispersion by conduction.

The sequence of two spike potentials is seen in the ventrolateral columns, whether the regions selected for recording be cervical, thoracic, lumbar or sacral, and whatever may be the conduction distance allowed. This fact has been confirmed with shocks delivered to the floor of the fourth ventricle and to all the regions of the spinal cord. Records obtained by employing a number of conduction distances in a single preparation (Fig. 1) indicate that there is a roughly linear relationship between the conduction distance and the ratio

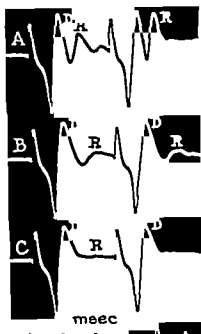


FIG 2 Thoracolumbar preparation Facilitation and asphyxial blocking of the synaptically relayed spike potential, R Two identical shocks were delivered to the ventral column of the lower thoracic cord Recording by microelectrode in lumbar ventral column Conduction distance, 9 cm

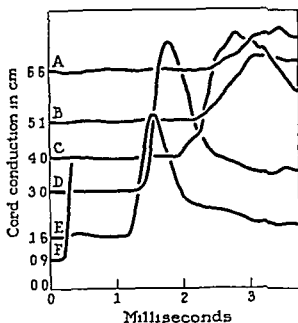


FIG 3 Response of the first sacral ventral root to ventrolateral column shocks Ordinates, distance of stimulating electrodes cranial to middle of first sacral segment in centimeters Abscissae, time from shock artifact in milliseconds The motoneuron responses were projected from the original records onto these coordinates and copied A, B, and C were recorded at $5 \times$ amplification for D, E, and F Hence responses are much smaller and less synchronous in A, B, and C

of synaptically relayed activity to unrelayed activity. Sectioning the neuraxis immediately cranial to the stimulating electrodes proves that the relayed spike potential must arise aborally from the stimulating electrodes. The relayed spike potential, furthermore, is recorded from the white substance itself, and is conducted in the same sense as is the initial spike potential. Hence the structures from which the second spike potential is recorded are propriospinal neurons, although when the stimulating electrodes are placed in the fourth ventricle, some reticulospinal elements may be contributing to the second spike potential.*

* The distinction between reticulospinal and propriospinal neurons is as artificial as is the division of the central nervous system into brain and spinal cord The retention of the distinctive terms is justified at the present time on the basis of common usage

There must be a fairly even distribution of the propriospinal somata along the long axis of the spinal cord to account for the regular increase with conduction distance in the relayed spike potential relative to the initial spike potential.

Emphasis must be placed on the fact that no critical evidence as to the axial length of the relay neurons can be drawn from experiments employing tract conduction. For instance, it would be possible theoretically to record both direct and relayed spike potentials with the conduction distance reduced to but a few millimeters, even though all the responding neurons had axial lengths of many centimeters. On the other hand, relatively satisfactory evidence has been obtained by recording the discharge of motoneurons in response to column shocks delivered at different distances from those motoneurons.

In Fig. 3 are shown the motoneuron discharges in response to ventral column shocks set up at several distances from the middle of a segment, the ventral root of which was used for recording. The individual records are arranged so that in effect cord conduction distance (ordinates) is plotted against latency of response (abscissae). In A, B, and C the amplification used was five times that for D, E, and F. The latency in F (0.2 msec.) is the ventral root conduction time, since the motoneurons are stimulated directly. In E there is a small direct response of the motoneurons, but the majority are now excited to discharge a nearly synchronous volley with a steplike additional latency of approximately 1.0 msec. This added latency is in a large part the synaptic delay at the motoneurons, though some cord conduction time must be included. In D, with 3 cm. cord conduction, the direct response is abolished, while the latency of the synaptically relayed response is increased to approximately 1.35 msec. The additional latency of D over that of E (0.15 msec.) accounts only for additional conduction time at the velocity of alpha fibers. Thus the responses shown in D, E, and F are similar in nature to the *M* and *S* waves recorded by Lorente de N6 from the oculomotor preparation (1939, p. 409, Fig. 4). The responses A and B are much smaller and less synchronous than those in D and E. A line drawn to join the points of onset of the motoneuron discharges in records A and B is closely parallel to a similar line joining the points of onset of the motoneuron discharges in records C and D, but it is later by approximately 0.7 msec. in the time axis. This is interpreted to mean that an additional neuron has been introduced into the shortest effective pathway to the motoneurons at conduction distances greater than 5 cm. In other experiments similar to the one detailed here the responses of the motoneurons with cord conduction distances greater than approximately 3.5 to 4 cm. have been still further delayed (for example, see Fig. 7A) or were even absent, unless they were preceded by another similar volley, in which case facilitation brought in a response with latency comparable to that of A and B of Fig. 3, but never comparable with that of D and E. The variability in unconditioned responses in different preparations is related to the general excitability of the individual preparations.

Record C of Fig. 3 illustrates an effect which has been encountered occasionally with conduction distances between 3 and 5 cm. The rising phase of the spike elevation has two parts. The onset of the main elevation, obtained by extrapolation of its rising phase down to zero potential, falls exactly on the plot of latency of responses A and B. The small initial elevation, partially fused with the main elevation, has a latency intermediate to those of B and D, and is closer to both than the minimal time allowable for a synaptic relay (Lorente de Nó, 1938a). Since it is not possible that the pathway for response B can be more than one neuron longer than that for D, the best available interpretation for intermediate latencies, appearing when a critical length of cord is traversed, is that they are the result of the summation of impulses in the slowest conducting pathways having one synaptic relay, with those in the fastest conducting pathways having two synaptic relays. Specific differences in individual synaptic delays within the relatively fixed limits established by Lorente de Nó (1938a) could aid in producing this effect.

Thus, when the stimulating electrodes are placed within 3 or 4 cm. of a motoneuron pool, the motoneurons discharge following the direct tract volley; when at longer distances the motoneurons discharge only after the arrival of the relayed tract volley at the pool. Therefore, for the alpha-tempo conduction pathways of the ventrolateral columns, leading to the motoneurons, the relay occurs at a mean distance of about 3.5 cm. These neurons intercalated in the shortest *effective* pathway to the motoneurons from more cranial regions of the spinal cord, or reticular formation, are obviously short propriospinal neurons (short spinal association neurons of Sherrington and Laslett, 1902, 1903). It must be noted here that the present method of indicating the axial length of the relay short propriospinal neurons demonstrates only the maximal distance at which sufficient of the shorter propriospinal neurons are excited directly to effect the discharge of motoneurons with only one synaptic delay. Therefore, it cannot be denied that many of the short propriospinal fibres will be shorter than this. On the other hand, it is shown below that much longer descending fibres enter into synaptic relation with the motoneurons. There is, therefore, no indication that the short propriospinal fibres occupy the position of specialized but true "Schaltzellen" in the sense of von Monakow (cf. Cajal, 1911, p. 150). Conversely, there is no experimental proof here that some do not.

It is unlikely from the direct evidence of tract conduction that many of the alpha pathways from superior to inferior regions of the spinal cord

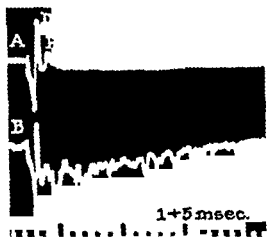


FIG. 4. Lumbar preparation. Comparison of white column and gray substance responses to a single ventrolateral column shock. A, recorded by microelectrode in ventral column; B, recorded by the same microelectrode placed in the ventral gray horn.

employ more than two axially orientated neurons lying within the white columns. Hence the typical large celled internuncial pathway may be considered as including a long propriospinal or bulbospinal neuron, which has feeble endings on motoneurons, with powerful collaterals to short propriospinal neurons. These latter have, in turn, endings on the motoneurons.

Response of the gray columns

While the activity of the ventrolateral white columns, as recorded by the microelectrode, stops abruptly following the completion of the double spike potential complex set up by a single shock, a microelectrode in the gray substance, by contrast, reveals a period of intense activity lasting some 15 to 20 msec. Figure 4 shows the recorded response with a microelectrode placed in the white column (A) and in the ventral horn of the gray substance (B). In each case the source of the excitation was a single shock to the column. The unmistakable localization of the burst of activity within the gray substance demands its origin from elements lying wholly within the gray substance. The elements contributing this burst must be Golgi type II cells constituting local or segmental interneuron pools. It is clear that the local interneuron pools are thrown into activity by the action of descending column fibers.

On repetition of the ventral column shocks, the activity within the local interneuron pool referable to subsequent shocks is facilitated during the first few msec., but it suffers occlusion and shortening thereafter. Figure 5 demonstrates this fractionation or channeling process in the interneuron pools accessible to column impulses. Three similar shocks were applied to the columns of the lower thoracic cord. The responses of the gray substance to these shocks in isolation are seen in Fig. 5 (A, B, and D). Figure 5C, in which two shocks fall in succession, shows the greatly synchronized response elicited by the second shock in comparison with its response in isolation seen in 5B. Figure 5E shows the response to a third shock similarly affected. By virtue of the channeling effect of combined facilitation and occlusion the actual internuncial response

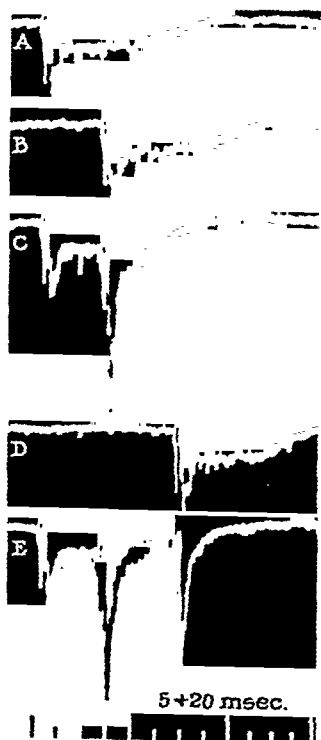


FIG. 5. Lumbar preparation. Response of the gray substance, recorded by microelectrode placed in ventral horn, to repeated stimulation of the ventrolateral column. A, B, and D are the responses to the three column shocks in isolation. In comparing C with A and B, and E with C and D, note channeling of activity through shorter interneuron chains by virtue of previous activation of the pool.

response to a third shock similarly affected. By virtue of the channeling effect of combined facilitation and occlusion the actual internuncial response

to three successive column shocks, seen in 5E, is qualitatively very different from that calculated by the simple addition of the isolated responses 5 (A, B, and D). If the discharge and facilitation of the motoneurons is referable to these interneuron pools, then the qualitative changes described here should be reflected by the motoneurons. As is shown below in Fig. 12, this is the case. Hence it may be said that the internuncial impulses initiated by the descending column fibers, in turn impinge upon the motoneurons.

Figure 6 is a diagrammatic representation of the intermediate spinal mechanism through which long reflexes, occupying alpha-tempo fibres, must act on motoneurons. The connections established between fibres of the ventrolateral columns, the segmental interneuron pools, and the motoneurons are

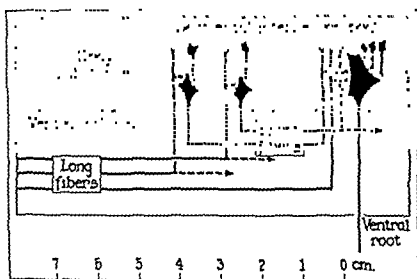


FIG. 6. Diagram of the pathways connecting the neurons of the ventrolateral column among themselves and with the motoneurons. Functionally the descending column system may be divided into groups of long and short fibers. The scale at the bottom indicates the approximate distance in centimeters of structures relative to the ventral root. For instance, the somata of the dominant short propriospinal neurons lie about 3 to 4 cm. cranial to the segment. The segmental interneuron pools are represented only by the final members of the chains. Paths indicated by broken lines are probable, but unproven.

shown. The pattern of the intermediate spinal mechanism as represented appears to be repeated at various levels of the cord, the position of any particular complex being a function of the position of a selected ventral root. All of the experiments to be discussed subsequently have been performed with conduction distances greater than the critical length of approximately 4 to 5 cm., and hence involve mediation of activity by the intermediate spinal mechanism as defined by Fig. 6.

Response of cord and motoneurons to a second tract shock

A single shock to the ventral column bundles discharges motoneurons either following the delivery of impulses relayed through the short propriospinal neurons, as in Fig. 3 (A and B), or after one or more additional

relays in the local interneuron pools. This is seen by comparing motoneuron latency in 7A (upper) with the time of the tract spikes recorded from the same segment in 7A (lower). The motoneuron discharge once instituted may last throughout the period of internuncial barrage.

On repetition of the ventral column shock, the short propriospinal impulses are increased by facilitation, as in 7 (C to H), lower records. These

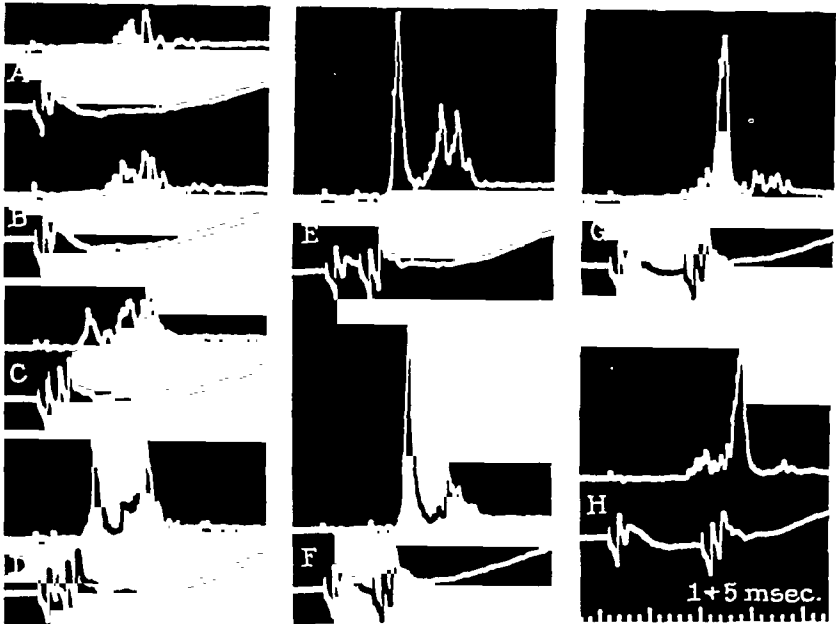


FIG. 7. Thoracolumbar preparation. Upper record in each of A to H is obtained from the 7th lumbar ventral root, lower record by microelectrode from the 7th lumbar segment. Cord conduction, 8 cm. A, response to a single column shock; B, to two shocks delivered simultaneously; C to H, to two shocks delivered serially at increasing intervals. Note synchronized motoneuron discharges produced by channeling of the internuncial response, and rigidly controlled in time by the relay short propriospinal volley.

fortified short propriospinal impulses now converge with local internuncial impulses at the motoneurons, with the result that the motoneuron discharge is advanced to a point but one synaptic delay behind the short propriospinal volley, as in 7C. The stimulus interval in 7C is 1 msec., while the motoneuron discharge (upper record) is advanced 3 msec. measured from the time at which it occurs following the conditioning shock alone (7A, upper) to a point just after the short propriospinal spike potential set up by the second shock (7A, lower). The short propriospinal impulses deliver a strong sharply synchronized synaptic excitation to the motoneurons, in contrast to the local internuncial barrage, which is highly asynchronous. Short propriospinal impulses from a second shock, converging with local interneuron pool im-

pulses, select a discrete segment of the motoneuron discharge for facilitation, with the result that a sharply synchronized motoneuron volley is produced, whereas the increase in local internuncial activity effected in 7B, by 2 simultaneous shocks, merely fortifies the asynchronous motoneuron discharge. The local interneuron pool impulses from the two shocks partially summate at the motoneurons when the shocks are seriatim, but again the resulting increase in motoneuron activity from this source, as in 7B with

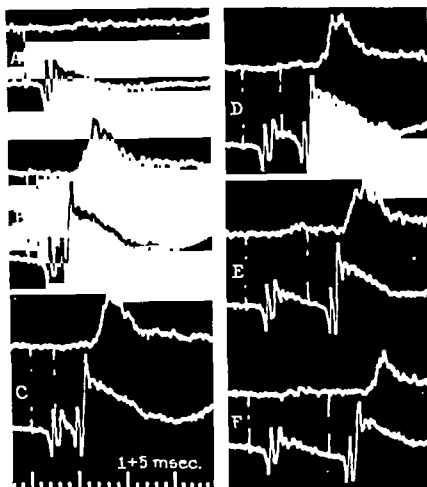


FIG. 8. Bulbosacral preparation. Cord conduction distance is 24.5 cm. Records from the 1st sacral ventral root above, and 1st sacral segment below, in each of A to F. Response to single shock to the floor of the fourth ventricle in A. B to F show responses to two similar shocks at increasing intervals. The onset of the facilitated motoneuron discharge is controlled in time by the short propriospinal volley.

simultaneous shocks, is much less intense and synchronous, as can be seen by noting the later motoneuron spikes in 7 (C to F). As the second shock falls still later (7, G and H), there is a marked decrease in the later motoneuron activity attributable to the second shock, although the facilitated synchronized volley is still in evidence. This decrease in motoneuron activity may be correlated with the occlusion of "cord potential" observable in the lower records of Fig. 7.

It may be noted now that the activity of local interneuron pools, as recorded by a microelectrode placed in the cord, manifests itself by a potential change of approximately 20 msec., during which period discrete spikes may

or may not be present. The prominence of spikes in such records is largely a matter of effective size of the recording microelectrode, the finer electrode favoring the recording of discrete spikes. The potential change, irrespective of electrical sign, is the analogue of the negative intermediary potential of Gasser and Graham (1933).

Figure 8 illustrates an experiment similar to that of Fig. 7. In this case, however, the shocks were delivered to the floor of the fourth ventricle. Records were obtained from the first sacral segment of the cord (lower records in each of 8, A to F) and the first sacral ventral root (upper records). A conduction distance of 24.5 cm. intervened. Practically no motoneuron discharge accompanies a single shock (8A, upper), although the cord record clearly reveals the direct and relayed spike potentials (8A, lower). With two shocks at various intervals (B to F), the relayed (propriospinal) impulses from the second shock are strongly facilitated, and as before with shorter conduction distance, they control the onset of the facilitated motoneuron discharge. With long conduction distance the higher multiple chains become relatively more effective, yet it is striking that the duration of the facilitated motoneuron discharge is not much more than doubled by the addition of 16.5 cm. of cord conduction.

The results of Fig. 8 emphasize the fact that the shortest effective pathway from the bulbar centers to the spinal motoneurons is through the propriospinal relay. It has not been possible to study the action of the long propriospinal fibers in isolation from the bulbospinal fibers. However, the essentially identical behavior of the system in Fig. 7, where a large portion of the fibers stimulated must be long propriospinal fibers, and in Fig. 8, where the long propriospinal fibers are certainly not stimulated, is strong evidence that the long propriospinal fibers play a part in relation to the spinal motoneurons more nearly like that of the bulbospinal fibers than that of the short propriospinal fibers.

Subliminal actions of ventral column volleys on motoneurons

It is possible to test the average excitability of a segmental population of motoneurons under any given set of circumstances by means of an ipsilateral dorsal root volley, while recording from the ventral root of the same segment (Renshaw, 1940). The first elevation of the reflex discharge set up by the dorsal root shock is mediated by arcs of two neurons, so that alterations in this elevation must reflect changes in the excitability of the motoneurons to synaptic stimulation, as a result of any chosen conditioning activity. In these experiments the conditioning activity is derived from shocks delivered to the ventral columns, or the floor of the fourth ventricle. The resulting alterations in the two-neuron arc reflex discharges demonstrate the subliminal changes associated with the motoneuron discharges described above.

Since with conduction distances greater than approximately 4 cm., the motoneuron discharge occurs only after the impulses of the short propriospinal relay impinge on the motoneurons, it is important to know whether

or not the long fibers mediating the direct spike potential volley of the cord record have synaptic connection with the motoneurons. Figure 9 illustrates an experiment designed to settle this question. A single shock was delivered to the ventral column bundles with the result shown in Fig. 9 (A and B). Figure 9B was recorded at 5 times the amplification used for the other records, to show details of the motoneuron response to the conditioning shock. A dorsal root volley in two neuron arcs, 9C, was caused to impinge on the motoneurons during the latent period of the response to the conditioning activity, with the result seen in 9 (D to L). By following the amplitude of the initial reflex discharge elevation, marked with an arrow, it will be noted that a maximum is reached in 9G, after which comes a decrease, 9H, and a

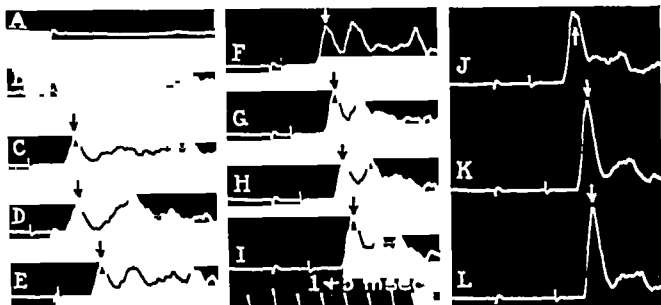


FIG. 9 Thoracolumbar preparation. Cord conduction distance 8 cm. Records obtained from 7th lumbar ventral root show facilitation of two neuron segmental reflex arc discharge by descending cord volleys. A and B response to single conditioning column shock in isolation. B at $5\times$ amplification of other records. C response to the dorsal root test shock in isolation. In the discharge mediated through two neuron arcs is the elevation identified by an arrow. In D to L the dorsal root shock falls progressively later. Note the bimodal facilitation of the two neuron arc discharge: the first maximum being reached in G

second increase, 9 (I to L). This effect is best seen graphically, as in Fig. 10A in which many more separate observations could be included. In Fig. 10A the amplitude of the testing two neuron reflex response is plotted as ordinate, against the interval between the conditioning ventral column shock and the testing dorsal root shock as abscissa. As demonstrated by Lorente de N  (1935b), summation between impulses in different pathways at the motoneuron is maximal when the shocks are simultaneous, and has a total duration of less than 0.5 msec, given negligible conduction and equality of paths. Some divergence from these rigid criteria is inescapable in the present experiments, since there is a marked inequality of paths. In Fig. 9 and 10 the column conduction is 8 cm, the dorsal root conduction is 2 cm. Because these conduction paths are not only unequal, but in addition are relatively long, there will also be a degree of temporal dispersion of the detonator

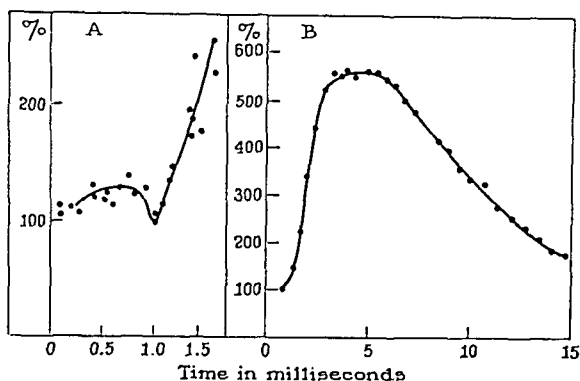


FIG. 10. Facilitation of motoneurons by a single column shock. Amplitude of the two-neuron arc response (ordinates) is plotted against the interval between conditioning column shock and testing dorsal root shock. Curve A presents detail of the early part of the facilitatory action of the column volleys and is constructed from observations, some of which are shown in Fig. 9. Curve B, the later course of the facilitation. Amplitude of the test response in isolation is 100 per cent.

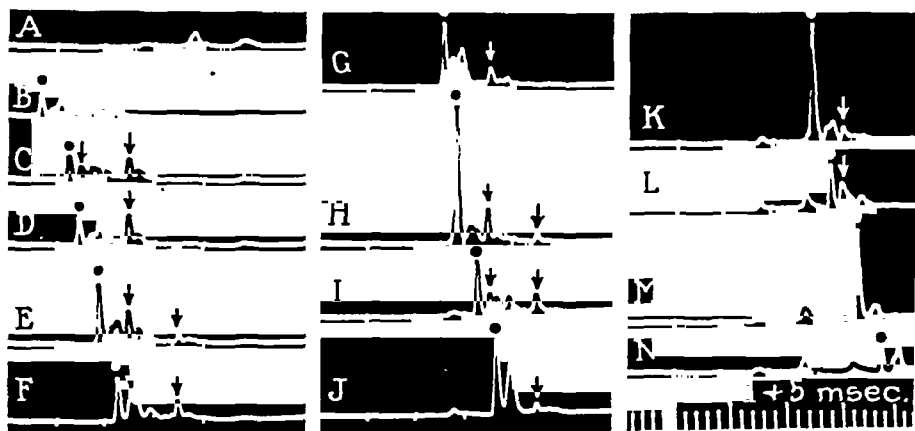


FIG. 11. Thoracolumbar preparation. Records from ventral root. Response of the motoneurons to four similar column shocks in succession shown in A; response to single dorsal root shock in B. The two-neuron arc elevation in B is identified by a dot. In C to N the dorsal root shock falls progressively later in the response to the four column shocks. The amplitude changes in the testing two-neuron arc discharge, marked throughout by a dot, reveal synchronization and augmentation of subliminal actions set up by the 2nd, 3rd, and 4th column shocks, which is not apparent following a single conditioning column shock (see Fig. 10B). Synchronization of subliminal actions on motoneurons parallels the synchronization of motoneuron discharges, both being due to channeling of activity through the shorter interneuron chains resulting from repetitive stimulation of the ventrolateral columns. Note also that the facilitated motoneuron discharge peaks resulting from convergence of later activity from the dorsal root shock with the column volleys and identified in the figure by arrows, are controlled in time by the direct column volleys, not the relayed short propriospinal volleys.

actions (Eccles) from both conditioning and testing sources. Given these somewhat unsatisfactory conditions, the fact that the first period of facilitation in Fig. 10A is distinct and has a total duration of less than 1 msec., is indisputable proof that long fibers do have synaptic connection with the motoneurons. The second rise in the facilitation curve, Fig. 10A, beginning in the 2nd msec., demonstrates the more powerful facilitating action of the short propriospinal relay impulses converging at the motoneurons. Subsequently facilitation progresses on a smoothly rising and falling course, 10B, which lasts during the period in which local internuncial activity is demonstrable, as in Fig. 4B, 5, and 7.

A correlation between the firing zone and the subliminal zone of a motoneuron pool can be shown under the conditions of repetitive excitation. Figure 11 presents records from an experiment in which the subliminal zone of the motoneuron pool is examined by means of changes in the two-neuron arc discharge as before. The response of the motoneurons to four similar column shocks in succession, seen in 11A, indicates directly the firing zone. The response to the testing dorsal root shock in isolation appears in 11B. In 11 (C to N) the test shock was caused to fall progressively later in the course of the response elicited by the conditioning column shocks. The two-neuron arc elevation is identified throughout by a dot. The course of facilitation, as indicated by amplitude changes of the two-neuron arc elevation, has four maxima, occurring in, E, H, K, and M. Each maximum occurs at the time of a motoneuron discharge in response to one of the four conditioning shocks.

Figure 12 shows a similar experiment in another preparation. A is a plot of the amplitude of the testing two-neuron arc response, and B shows, on the same time scale as used in the plot, the motoneuron response to the conditioning excitation, which is three shocks to the ventral column. As in Fig. 11, a peak of facilitation corresponds to each motoneuron discharge. The onset of each peak of facilitation is as rigidly fixed in time with respect to the causal shocks as is any known feature of synaptic activity. The form of

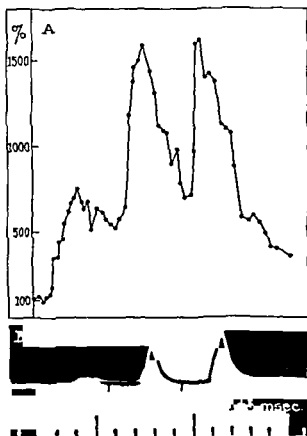


FIG 12. Facilitation of motoneurons by three successive column shocks, the response to which in isolation is shown in B. Curve A plots the amplitude changes in the testing two-neuron arc volley as in Fig 10. The course of the subliminal actions on motoneurons reflects the channeling of internuncial activity as recorded in Fig 5. Frequency of stimulation for this effect is not critical, the only requirement being that subsequent shocks fall during the period of internuncial activity initiated by antecedent shocks.

the action potential of the motoneuron discharge (12B) is determined by the number and temporal distribution of the motoneurons that are fired. After each added shock the number of motoneurons fired is increased and the firing occurs in greater synchronization, in exact accord with the prediction from the augmentation and synchronization of the internuncial activity that was described in connection with Fig. 5. In the corresponding facilitation curve (12A) it will be noted that the peaks change in form in a manner similar to that of the elevations in the motoneuron discharge record. They also become higher and shorter. Thus there can hardly be any doubt that the facilitation is caused by a bombardment of the motoneurons in the subliminal fringe by the same group of internuncial impulses that controls the supraliminal excitation.

Activity in dorsal and ventral interneuron pools in relation to effectiveness of column volleys

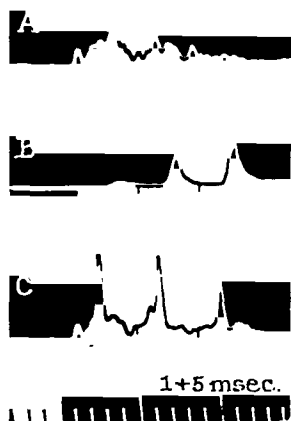


FIG. 13. Lumbar preparation. Conduction distance, 4.5 cm. A, motoneuron response to a single dorsal root volley. B, motoneuron response to three column shocks. The motoneuron discharge volleys in B follow the arrival of the short propriospinal relay impulses at the motoneurons. In C where the column shocks fall during the course of the response to the dorsal root shock, the direct column volleys control the time of the facilitated motoneuron discharge peaks.

It is a fact repeatedly confirmed that a volley of impulses arriving at the motoneurons by the direct column fibers cannot effectively discharge these motoneurons, even if they converge with impulses from interneuron pools activated by other column volleys. On the other hand, when the same volley of impulses in the direct fibers converges with impulses from interneuron pools activated by a dorsal root shock, they are effective. Figure 13A shows the motoneuron response to a single dorsal root shock. Following the two-neuron arc elevation there is a random discharge lasting some 11 to 12 msec. Figure 13B shows the motoneuron response to three similar successive ventral column shocks. The three elevations of 13B appear following the arrival of the relayed column impulses in each case. In 13C the dorsal root and ventral column shocks are combined, with the result that three elevations, synchronized to 1 msec, or less, appear approximately 0.8 msec. in advance of the position of the corresponding elevations in 13B. In over 70 individual observations on this preparation no intermediate latencies were encountered. The control of the motoneuron response was advanced in a step-like manner from the relayed column (short propriospinal) impulses to the direct column impulses. Figure 11 shows the same effect in another experiment. The preparation in this case was narcotized

with dial rather than decerebrated. The random discharge resulting from the single dorsal root volley lasted only 7 to 8 msec., as seen in 11B. As the

dorsal root shock is progressively delayed, 11 (C to L), it may be noted that sharply synchronized peaks appear out of the random reflex discharge as the latter encroaches upon the time of arrival of the successive direct column impulses at the motoneurons. These peaks are marked by arrows. The time at which these peaks appear is constant, being approximately 1 msec. in advance of the peaks elicited by the column shocks acting alone, and it is determined by the arrival of the direct column impulses. The absolute difference in latency between the observations of Fig. 11 and 13, approximately 1 msec., is occasioned by the different cord conduction distance used in the two experiments.

It is certain from anatomical studies (Cajal, 1909, Fig. 126) that separate interneuron pools are available to impulses from the tract fibers, and to impulses from dorsal root fibers. The former lie in the ventral horn region, the latter in the dorsal horn and the intermediate regions. Impulses from the ventral pools converging with the relayed tract impulses are effective, while those converging with the direct tract impulses are not effective. On the other hand, impulses from the dorsal pools converging with the direct tract impulses are able to excite motoneurons. This differential exciting power may result from a specific arrangement of synapses from the two groups of pools (see Lorente de Nó, 1938b), or it may merely indicate quantitative differences in their impulse output. At the present time there is neither the anatomical equipment nor an adequate differential measure of pool activity with which to carry reasoning further to a decisive answer.

Influence of other systems on propriospinal nuclei

The corticospinal system. Stimulation of the motor cortex conditions the discharge of propriospinal nuclei at various levels of the spinal cord; two examples are shown in Fig. 14. Figure 14A is the response of the cervical ventral column to a single shock delivered to the floor of the fourth ventricle. In 14B the floor shock is preceded by a train of 5 shocks delivered to the motor cortex. The direct spike potential of the column response is slightly increased, which may be interpreted as the result of summation at the reticular neurons of synaptic stimulation, mediated by corticobulbar fibers, and electrical stimulation by the floor shock (Lorente de Nó, 1935c). More prominent, however, is the facilitation of the relayed spike potential of the cervical short propriospinal neurons. Figure 14 (C, D, and E) demonstrates a similar effect of corticospinal activity on propriospinal neurons, the somata of which lie in the lumbar cord. Figure 14C shows the response of the lower lumbar cord to 5 shocks delivered to the motor cortex, while D is the response to a single shock applied to the ventral column of the upper lumbar cord. Under these experimental conditions the propriospinal relay must lie in the lumbar cord. Figure 14E, in which the lumbar cord shock is preceded by the motor cortex shocks, demonstrates that the response of the lumbar propriospinal neurons is facilitated.

The latency of the corticospinal effect is long, varying from 5 to 15 msec. by direct observation. One factor in the duration of the latent period is the remoteness of the spinal field chosen for study. In addition it is possible that the first detectable influence is exerted not directly, but indirectly through interneuron pools.

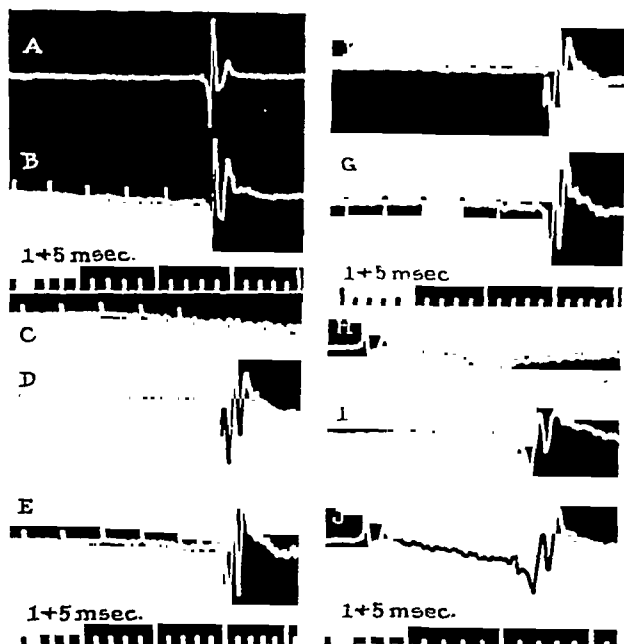


FIG. 14. A and B, bulbocervical preparation. A, response of the ventrolateral column of the cervical cord to a single shock to the floor of the fourth ventricle. In B, the propriospinal relay spike discharge of this response is facilitated by the action of 5 shocks delivered to the contralateral motor cortex. C, D, and E, lumbar preparation. C, response of the lumbar cord to 5 shocks delivered to the contralateral motor cortex. D, response of the lumbar cord to a column volley delivered to the upper lumbar region. In E, the lumbar propriospinal spike discharge is facilitated by the action of the cortical volleys. F and G, lumbar preparation. The propriospinal relay of the column response seen in isolation in F is facilitated in G by the action of 5 shocks applied to the central ends of cut strands of the brachial plexus. H, I, J, lumbosacral preparation. H, response of the ventrolateral column to a single shock to the 5th lumbar root. Note that short propriospinal neurons are discharged by the dorsal root volley in H. In J, the discharge of the lumbosacral short propriospinal neurons is facilitated by the 5th dorsal root shock. I, column response in isolation.

Primary afferent systems. The influences of primary afferent neurons of the spinal region may be divided into two categories, which correspond to the long spinal reflexes and the short spinal reflexes of Sherrington (1898). Afferent neurons of the upper spinal field have an influence on lumbar propriospinal nuclei which, in its quantitative aspects, resembles that of the corticospinal system as noted above. Figure 14F is the response of the lower

lumbar cord to a single shock applied to the ventral column of the upper lumbar cord. This response is conditioned, in 14G, by 5 shocks delivered to the central end of strands of the severed brachial plexus, with the result that the spike potential of the lumbar propriospinal relay is facilitated. This effect on lumbar propriospinal neurons must involve at least the relay of activity through the long propriospinal neurons.

The results of stimulating primary afferent neurons, which supply the region of the short propriospinal relay itself, are somewhat different. The conditions for observing this situation were achieved in the experiment illustrated in Fig. 14 (H, I, and J) by placing the recording microelectrode in the first sacral segment and applying a ventral column shock 9 cm. orally. For conditioning, a single shock was delivered to the 5th lumbar dorsal root, the response to which is shown in 14H. Units of the propriospinal nuclei appear to be discharged directly by the dorsal root impulses, as shown by the presence of a relayed column spike potential following the dorsal root shock. Figure 14I shows the response to the testing ventral column shock in isolation. In Fig. 14J the ventral column shock is preceded by the 5th lumbar dorsal root shock, with the result that the discharge of the short propriospinal neurons is facilitated. A quantitative comparison may be drawn between the action of neighboring dorsal root fibers on short propriospinal nuclei, as in Fig. 14J, and the action of long propriospinal fibers on short propriospinal nuclei, as in Fig. 7H (lower record).

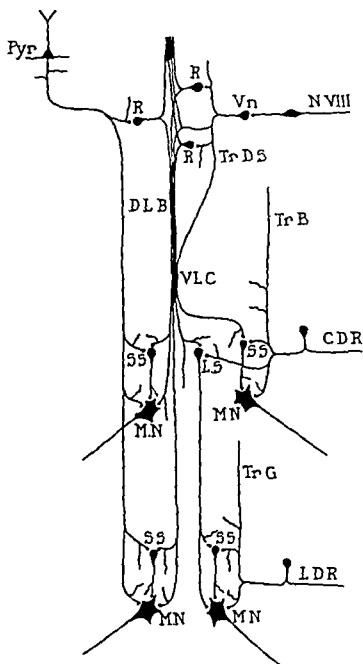


FIG 15 Functional organization of unilateral connections of the bulbospinal correlation system. The connections of various primary afferent systems are given on the right-hand side. Connections from the contralateral motor cortex are on the left hand side. CDR, cervical dorsal roots, DLB, dorsal longitudinal bundle, LDR, lumbar dorsal roots, LS, long propriospinal neurons, MN, motoneurons, N VIII, vestibular nerve, Pyr, corticospinal neurons, R, cells of the reticular formation, SS, short propriospinal neurons, Tr B, tract of Brachach, Tr DS, trigeminal tract, Tr G, tract of Goll, VLC, ventrolateral column, Vn, primary vestibular nuclei.

Functional organization of the correlation systems

Figure 15 serves as a diagrammatic survey of many of the foregoing observations and represents the functional organization of the correlation systems as they appear from the results of these experiments. It is admittedly incomplete and imperfect. Since no account has been taken of conduction across or up the spinal cord in these experiments, Fig. 15 includes only unilateral descending connections: the spinal pathways of least resistance (Sherrington, 1898).

It will be apparent from the foregoing experimental results that, with the exception of short spinal reflexes (Sherrington), all of the bulbospinal and descending spinal reflexes which employ the low threshold, rapidly conducting fibers, must involve two axially orientated neurons, one of which is a short propriospinal neuron. By comparing activity in the descending system with that of the ascending system to the oculomotor nuclei (Lorente de Nó, 1933-1939), it is clear that the propriospinal nuclei may be homologized with the reticular nuclei of the medulla, pons, and midbrain. Together the reticular and propriospinal nuclei form practically a continuous system of correlation centers influencing motoneurons from the oculomotor nuclei to the caudal spinal cord.

The correlation system is subjected to many external influences. The secondary vestibular fibers, for instance, are arranged so as to deliver impulses into the correlation system at all anatomical levels, through the dorsal longitudinal bundle, vestibulomesencephalic tract, and the vestibulospinal tract. Primary afferent systems other than vestibular send impulses into the system at their appropriate levels. The corticospinal system has been found to exert an influence at all levels. Although the vestibular and cortical influences enter the correlation system at all levels, this system must not be regarded simply as a collection of short fiber relays subservient to these influences at various levels; for within the system itself there are the long fibers of the reticulospinal tracts and of the long propriospinal tracts. The latter, by virtue of their demonstrated alpha conduction rate, must ensure the rapid diffusion of activity throughout the whole system. Thus it is possible that impulses in voluntary motor paths, through the known relays into the correlation system at the reticular formation, could assist in adjusting the posture of the whole animal to a state appropriate to the initiation of voluntary movement at the time of arrival of the voluntary motor impulses at the motoneurons.

SUMMARY

The bulbospinal correlation system may be divided into two fiber groups, the long fibers represented by the reticulospinal tracts and long propriospinal tracts, and the short fibers represented by the short propriospinal tracts. Activity traverses the length of the neuraxis in the long fibers and enters the short propriospinal nuclei, apparently at all levels, to travel a short distance as relayed impulses along the short propriospinal fibers.

Impulses from the long fibers reach the spinal motoneurons directly and by the appropriate short propriospinal relays. These tract impulses also activate local interneuron pools of the ventral horn region, which in turn deliver impulses to the motoneurons.

In activity initiated from the upper cord and medulla the summation of impulses in the short propriospinal relays with local internuncial impulses from the ventral interneuron pools controls the time of firing of the earliest motoneuron volleys. The direct tract impulses, arriving at the motoneurons approximately 1 msec earlier than the propriospinal relays, are ineffective. For the direct impulses to become effective the dorsal interneuron pools must be active. These dorsal interneuron pools are under the control of primary afferent neurons. When these pools are activated by a dorsal root volley, temporal control of the volleys in the motoneuron discharge passes from the relayed tract impulses to the direct tract impulses.

The total motoneuron discharge is in strict parallelism with the activity of intimately related interneuron pools. With repetitive volleys descending the cord, the internuncial activity is synchronized and augmented, and the activity so changed determines the size and duration of the motoneuron discharge. The subliminal fringe within the motoneuron pool varies with the discharge zone of that pool.

The propriospinal nuclei are subject to vestibular, reticular, corticospinal and primary afferent influences. They are, therefore, correlation centers in as true a sense as is the reticular formation.

The temporal exactitude with which discharges occur in the interneurons of the column tracts and in the motoneurons guided by these discharges, attests to the importance of timing as a factor in synaptic transmission in the spinal cord.

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DEVELOPMENT OF SWIMMING AND RIGHTING REFLEXES IN FROG (*RANA GUENTHERI*): EFFECTS THEREON OF TRANSECTION OF CENTRAL NERVOUS SYSTEM BEFORE HATCHING

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(Received for publication September 25, 1940)

FOR THE past twenty years, the study of the behavior of the embryo of different species of vertebrates has been aimed mainly at tracing the stages of behavioral development and correlating them with those of the ontogeny of the central nervous system (CNS), the sensory apparatus and the effector organs. Except the experiments of Detwiler (1927, 1933) on the extirpation of the Mauthner's cells in relation to reflex activities in *Amblystoma* and the observations of Hooker and Nicholas (1930) on the reflexes of rat fetuses with the spinal cord severed at the dorsal segments, the surgical technique has seldom been resorted to in unravelling the nervous mechanisms underlying the development of behavior. The surgical technique in the hands of numerous neurophysiologists has given a large amount of knowledge concerning the functions of the different parts of the adult CNS of different vertebrates. This knowledge should form the foundation for the analysis of the nervous mechanisms underlying behavioral development. And it should be of considerable interest to compare the physiological effects of the same operation on the brain or the spinal cord in the adult with those in the embryo of the same species of *Vertebrata*. Such comparison may throw light on the physiology of the developing CNS.

With these purposes in view we have performed a series of experiments with the frog tadpoles to find out the effects of transection of the CNS at different levels at approximately the stage of gill-buds on the development of one aspect each of locomotion and posture, swimming and righting reflexes. The results of these experiments are here reported.

METHODS

Our experiments were carried out with the larvae of the frog *Rana guntheri*, the toad *Bufo bufo asiaticus*, and *Microhyla ornata*. Most of our results were obtained with tadpoles of the frog, and therefore our report will be restricted to this species only. However, we would like to mention that similar results to those presented in this paper were observed in the larvae of the toad and microhyla.

The frog eggs were collected from ponds near Kweilin in the three months from May to August of 1938, and 1940.

In each series of experiments, 50 or 100 embryos were used and equally divided into 5 groups. With the embryos in group 1, a lesion was made on the neural tube at such a level as would produce a complete severance of the cervical cord in the larvae, with those of groups 2, 3 and 4, at such a level as would produce in the larvae a complete transection of the CNS at the rostral border of the hindbrain, the midbrain and the interbrain respectively, while the embryos of group 5 underwent no operation and served as controls. Altogether 10 such series were carried out.

The operations on the neural tube were performed with embryos about at the stage of gill-buds when they were still non-motile. Each embryo was shelled out of the egg, washed twice in water which had been previously boiled and cooled down to room temperature (circa 20°C.), subjected to operation on a sterilized slide under a dissecting microscope, with two sterilized needles as the cutting instruments, and thereafter transferred to a dish of boiled and cooled water. No anesthetic was used. The death rate due to operation was about 10 per cent. In the transection of the neural tube, a single section was insufficient to ensure the success of the intended operation, and it was necessary to tease out a strip

of tissue after the incision was made. The success of our operations was about 60 per cent. In our preliminary experiments we employed a cautery for producing lesions on the neural tube, and found that without rigid and fine control of the temperature of the cautery it caused too extensive destruction of the tissues around the site of operation.

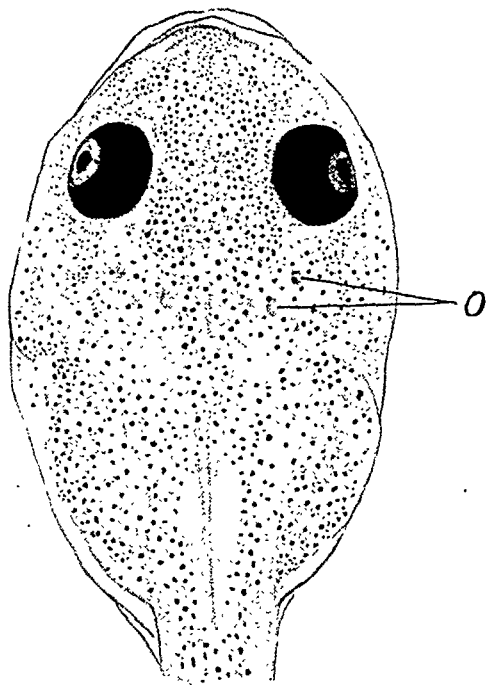
In some experiments carried out for the analysis of the results obtained, we amputated the tail and removed the labyrinths in a number of tadpoles. For labyrinthectomy we employed the following technique: A larva was immobilized by placing it in a mixture of water and ethyl alcohol for several minutes, and then laid on a sterilized slide under a dissecting microscope. The otoliths are visible as two dark spots on each side of the hindbrain (see Fig. 1). They and their adjacent tissues were easily destroyed with a needle. For amputation of the tail we simply incised it with a pair of scissors. No narcotic was used, and the wound was not closed, but care was taken to avoid the infliction of injury on the cloaca.

The larvae of *Rana güntheri*, when reared in a white porcelain dish, are light orange in color, and the outlines of the brain remain visible to the naked eye and under the microscope for over a fortnight

FIG. 1. Camera lucida drawing showing the outlines of the living brain of the larva of *Rana güntheri*. $\times 20$ diameters. O = otoliths.

(see Fig. 1). Frequently we could see under the microscope the continual rostral and caudal movements of a black particle in the fourth ventricle, indicating the existence of pulsation in the cerebrospinal fluid. Such enduring visibility of the outlines of the living brain proved a great advantage to us, working in a country at war where the abnormally high cost and the wellnigh inaccessibility of imported chemicals made it impossible for us to make preparations of the operated brain for microscopic examination. It enabled us to examine the operated living brain in each case as many times as necessary for determining the extent of the lesion made. In 10 cases these observations on the living larvae were controlled by dissections of brains after they were sacrificed and fixed in a 10 per cent aqueous solution of commercial formaldehyde for 3 or 4 days. The dissections done by Mr. H. T. Chang of this Institute who had not been informed about the sites of the lesions and the findings of behavioral observations confirmed the results of biopsy.

For tactile stimulation of the tadpole a horse hair securely tied onto a bamboo twig was used. This simple implement could be employed to put the larva on its back or side for a test of the presence of righting reflexes. For such a test we also adopted another method. We sucked a tadpole into the barrel of a medicine dropper, slowly turned the



dropper on the horizontal body axis of the tadpole therein and observed whether it would show any compensatory movements.

The body length of the tadpoles under experiment were measured to 0.1 mm. by means of a vernier caliper. The larva to be measured was placed on a slide and the caliper was applied to the larva underneath it. The accuracy of this procedure cannot be high. We controlled neither the food given to the tadpoles nor the temperature of their environment. Our measurements of body length served merely as a rough index of the growth of the tadpoles used.

Our observations of the behavior of the normal and operated larvae were continued up to 10 days after the gills were completely covered up by the operculum on both sides. By this time swimming and righting reflexes have already reached full development; we therefore deemed it unnecessary to follow it to the time of metamorphosis.

RESULTS

Our results may be best described under the following headings.

A (1) *The normal development of swimming*: Coghill (1929) divided the development of swimming in *Amblystoma* into 5 stages: (i) the non-motile stage, (ii) the early flexure stage, (iii) the coil stage, (iv) the stage of the 'S' reaction and (v) the stage of aquatic locomotion. Youngstrom (1938) found similar stages in the ontogeny of aquatic locomotion in different species of *Anura*. According to our observations, *Rana güntheri* showed similar stages, except the coil stage. We have never found the embryo of this species of *Anura* in a tight coil.

However, we should like to point out that it may be better to divide the 5th stage of Coghill into 3 stages: namely, (v) the stage of translatory movements of the body, in which the larva performs the 'S' reactions in sufficiently quick successions to effect locomotion; (vi) the stage of the control of the direction of the translation of the body, in which it not only effects locomotion but also is able to avoid obstacles and to change the direction of motion whenever necessary; and (vii) the stage of the active maintenance of normal spatial orientation, in which it actively resists any attempt on the part of the investigator to put it on its back or side while it is swimming.

The last 2 stages were found to be easily affected by lesions on the CNS caudal to the diencephalon. The sixth stage was reached in *Rana güntheri* at a body length of 5 to 6 mm., and the seventh at a body length of about 9.5 mm. Here it should be mentioned that a tadpole of less than 9.5 mm. in body length always gained the normal orientation to the earth's gravitation field whenever it started to swim. This was found to be entirely due to the position of the center of gravity in the body, because any deformity of the body which displaced its mass center from its normal position would, as will be shown later, make these larvae swim on their back or side. At and after the seventh stage of the development of aquatic locomotion, a larva actively kept the dorsoventral axis of the body pointing to the center of the earth, although it could swim on its back or side when such a position of the body was required for securing food. Only after this stage was reached, could a tadpole be regarded as having acquired the ability of swimming, if the word 'swimming' is not taken to mean merely the translatory movements of the body.

We also wish to call attention to the fact that the tail is the only prime mover in the aquatic locomotion of the tadpole. We amputated tails of over 10 normal larvae, and found that a tailless larva was utterly powerless to change its position even a little on the bottom of the dish, not to mention to swim. The tail remained, indeed, the only motor organ in the early larval life.

(2) *The effects thereon of the transection of the CNS at different levels:* The complete severance of the cervical cord (section 1 in Fig. 2) prevented the operated larvae from reaching the final two stages of the development of aquatic locomotion. For 1 or 2 days after the operation, they showed no abnormality in behavior. Then it was noticed that the tail bent ventrad whenever it moved, presumably due to the exaggeration of the contraction of its flexors over that of its extensors. The spinal column then also showed ventrad flexion. The ventroflexion of the tail and the spinal column grew more and more marked every day and finally became a permanent posture. The body of the spinal tadpoles then assumed the form of a ring with a ventral opening of various sizes according as their spinal columns and tails were more or less ventrad flexed. As a consequence of this deformity, the center of gravity of the body was displaced to a more ventral position on its dorsoventral axis, and this displacement caused the larva to lie on its side while at rest. Whenever it attempted to move, it swam on its side in a circle: clockwise, in case it lay on its left side; and counter-clockwise, if on its right side. It could neither control the direction nor maintain normal spatial orientation while swimming.

In a number of larvae the transection of the spinal cord was unintentionally made caudad to the cervical region, and with 20 others the spinal cord was purposely severed at the dorsal or the lumbar segments. In all of them a permanently ventrad flexed tail resulted and the development of aquatic locomotion was arrested at the stage of translatory movements of the body. In order to prove that this arrest of the development of swimming is caused not by the deformation of the body but by the severance of the connections between the cord and the higher centers, we sought for a method of producing a ventrad curved tail without any injury to the CNS. By accident we found that such a tail was induced by several incisions suitably placed on the ventral side of the tail. This operation was performed on 10 normal larvae and observed that the ventrad curved tail hampered their swimming greatly but by no means prevented them from reaching the full development of aquatic locomotion. Moreover, we found that in one spinal tadpole the severed cervical cord became re-united 5 days after the operation leaving no visible trace of the original lesion either to the naked eye or under the microscope. This larva first showed the exaggeration of the flexor reflexes of the tail. Then the exaggeration of the flexor reflexes became decreased instead of increased in intensity, and finally disappeared. After this transient phase of accentuated flexor reflexes, the larva developed behaviorally just as a normal one.

The transection of the CNS at the caudal border of the midbrain (section 2 in Fig. 2) prevented the operated tadpoles from attaining the ability of maintaining normal spatial orientation during swimming. During the first day after the operation, the behavioral development did not deviate from its normal course. Then a gradual change in the posture of the tail occurred: it became permanently dorsiflexed. This is a phenomenon similar to the decerebrate rigidity of adult mammals, an over-reaction of the antigravity muscles. The extension of the tail varied widely in intensity from one decerebrated larva to another: from being barely recognizable to being so marked that the dorsiflexed tail made an angle of over 10 degrees with its base. Such extreme dorsiflexion of the tail displaced the mass center of the body to a more dorsal position on its dorsiventral axis. Consequently the tadpole lay partly on its side and partly on its back during rest, and swam on its back. It could, while swimming, alter its course in case this was necessary; but it never attempted to correct the *reversed* orientation to the earth's gravitation field. Even in those decerebrated larvae whose extension of the tail was not so marked, no active resistance was observed to the attempt on the part of the investigator to put it on its back or side while it was swimming.

The transection of the CNS was accidentally effected in a number of larvae at various places in the caudal two thirds of the hindbrain, and this operation was found to have the same effects on the development of aquatic locomotion as complete severance of the spinal cord. With five embryos the neural tube was transected at the level of or below the ear vesicles, and they all showed the same symptoms as the spinal tadpoles. All these observations justify the conclusion that the area in the hindbrain responsible for the control of aquatic locomotion is located in its rostral one third. From the structure of the medulla oblongata (Ariëns Kappers, Huber and Crosby, 1936), we may assume that this area corresponds either to the vestibular nuclei or to the reticular elements in the rostral one third of the hindbrain. There is no experimental evidence supporting either of these two alternatives.

On 10 decerebrated tadpoles we performed double labyrinthectomy. They all lost the control of the direction of aquatic locomotion, turning somersaults in every attempt at swimming. And they did not recover from this effect. These experiments certainly showed the importance of the impulses from the proprioceptors in the labyrinth for the control of the direction of swimming. In numerous normal and decerebrated larvae we found that the removal of both labyrinths without injury to the adjacent brain induced no exaggeration of flexor reflexes and no consequent deformation of the tail and the spinal column. This observation suggests that the impulses from the somæsthetic sense organs, including the proprioceptors in the musculature, play a large rôle in the control of reflex activities of the spinal cord by the motor center in the medulla oblongata.

The transection of the CNS rostral to the rostral border of the midbrain (section 3 and 4 in Fig. 2) produced no effects whatsoever on the development of aquatic locomotion. The findings on the thalamic and mesencephalic

tadpoles, together with the changes observed in swimming after decerebration and severance of the spinal cord, located the highest center for aquatic locomotion of the tadpole in the mesencephalon. On account of the lack of facilities for making series sections of the operated larvae, we did not perform any experiment to further limit the location of this center. However, we may from many incidental observations venture the conjecture that it probably lies in the caudal half of the tegmentum.

We found that the unintentional extirpation of the tectum did not arrest the full development of swimming, and that the accidental removal of the rostral half of the tegmentum together with the optic lobes also was without any effect.

In this connection it should be mentioned that all the operations on the CNS did not retard the growth of the larvae, except the transection of the spinal cord which delayed it for 2 days. The tadpoles with the cervical cord severed were found to be difficult to rear. They invariably developed the communicating type of internal hydrocephalus. All the ventricles in the brain became greatly dilated; the puncture of the forebrain to release the accumulated cerebrospinal fluid brought about relief for at most 24 hours or even less. The loss of the ability of free swimming also gave them many difficulties in obtaining food. They survived the operation for less than 10 days.

B (1) The development of the righting reflexes: From the results presented in the preceding paragraphs it is evident that the righting reflexes developed rather late. They were observed to appear in the interval between the full growth of the gills and that of the operculum (body length from 6.5 to 9.5 mm.). The exact time of first appearance of these reflexes was very difficult to determine. The gills floating on both sides of the body acted as a

kind of balancing organ, and mechanically assisted to a great extent the tadpole in keeping normal spatial orientation. When put on its back, it would right itself up with the assistance of the floating gills by any chance movement. When drawn into the barrel of a medicine dropper for observations on its compensatory movements to slow rotation of its body on the horizontal axis, it moved every time the gills came by chance into contact with the wall of the dropper; which was very difficult to avoid. However, repeated observations on a large number of larvae convinced us that they acquired the righting reflexes in the time interval stated above.

The time of first appearance of the righting reflexes was found to be later

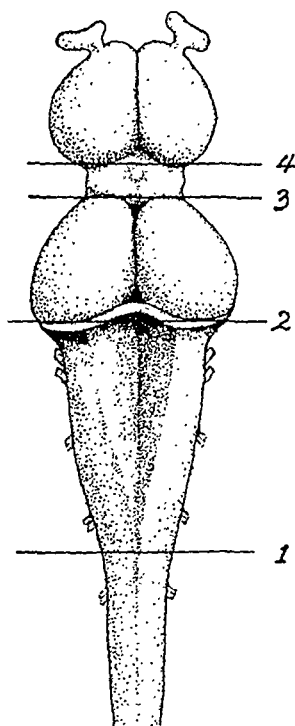


FIG. 2. Drawing of the dorsal view of the larval brain of *Rana güntheri* showing the four sections used in our experiments.

than that of the coming into function of the proprioceptors in the labyrinth. With tadpoles of 6 mm. body length which had not as yet developed the righting reflexes, the ablation of both labyrinths produced the usual effects on locomotion: these larvae entirely lost the control of the direction of aquatic locomotion and turned somersaults whenever they attempted to swim.

(2) *The effects thereon of the transection of the CNS at different levels:* The transection of the CNS caudal to the rostral border of the hindbrain (sections 1 and 2 in Fig. 2) suppressed the development of the righting reflexes in the tadpoles, while the extirpation of the forebrain and the interbrain or of the telencephalon alone (sections 3 and 4 in Fig. 2) neither suppressed nor retarded the development of these reflexes. That the centre for the righting reflexes lies in the midbrain in the larva just as in the adult frog, found support in the following experiments. The destruction of the midbrain of a mesencephalic tadpole immediately abolished its ability of maintaining the normal orientation to the earth's gravitation field. Furthermore, we found that normal, thalamic and mesencephalic larvae of *Rana guntheri* could recover from the effects of double labyrinthectomy, provided that there was no concomitant injury to the hindbrain. On the extirpation of the mesencephalon in all these tadpoles the symptoms of double labyrinthectomy immediately returned, as did the loss of the righting reflexes and of the control of the direction of swimming. We have already stated that the decerebrated larvae never recovered from the effects of the removal of both labyrinths.

DISCUSSION

From the work of Coghill (1914-1936, 1929) and Herrick (1937, 1938) on the correlation of the behavioral with the anatomical development in *Amblystoma*, the following facts are known. The growth of the muscles and the somaesthetic sense organs precedes that of the spinal cord. In the non-motile stage the relative growth rate is the highest in the spinal and the post-facial region of the CNS, and from the stage of the early flexure to that of early swimming, in the post-facial region alone. The growth of the mesencephalon and the diencephalon begins to increase in rate at the 'S' reaction stage. No work along these lines has been carried out on the ontogeny of the CNS in *Rana*, and the observations by Coghill on the late stages of the development of swimming in *Amblystoma* are not comparable with our results on the frog. If it is justifiable to combine the facts discovered by Coghill and Herrick and our results, we may conclude that the maturation of the muscles and the somaesthetic sense organs and the spinal cord prepares for the advent of the stage of the transitory movements in the development of aquatic locomotion; that the full growth of the hindbrain antedates the stage of the control of the direction of swimming; and that the full development of the midbrain precedes the stage of the maintenance of normal spatial orientation during motion and rest. In short, each particular stage in the ontogeny of behavior follows maturation of the nervous mechanisms con-

cerned, which is, in turn, preceded by that of the receptor and effector organs involved. This conclusion serves well as a generalization of the relations between the behavioral and the anatomical development in Amphibia.

Comparison of the normal development of swimming with the development after transection of the CNS at different levels in the frog brings out the following important fact. Although the severance of the spinal cord induces exaggeration of the flexor reflexes of the tail and decerebration enhances its extensor reflexes, yet no such phenomena have been encountered in the normal ontogeny of aquatic locomotion. From the fact that the maturation of the CNS proceeds *von unten nach oben*, we certainly expect to find them. The explanation of this fact is found in the following data discovered by Coghill and Herrick in *Amblystoma*. The accelerated growth of the medulla oblongata starts simultaneously with that of the spinal cord and outlasts it; while the development of the diencephalon and mesencephalon begins to increase in rate before the maturation of the hindbrain. The neurons in the higher centres send their axons caudad to the lower centres, before they have acquired functional connections with the incoming sensory and other tracts. Thus, the descending fibres from the neurons in the higher centres may, as suggested by Herrick (1938), exert some influences on the spinal centres at a time when the neurons have not yet assumed full function on account of the lack of the necessary connections with the incoming tracts. This seems quite possible, as we now know from the numerous investigations carried out in recent years on the electric activity of ganglionic centres (Davis, 1939) that the neurons are not merely passive tools in the hands of the receptor organs, but agents capable of spontaneous activity.

The findings of our experiments on the effects of transection of the CNS at different levels upon the development of aquatic locomotion and righting reflexes generally agree with the results of previous works on the changes in behavior after the same lesions on the brain and spinal cord in adult amphibians and mammals, including man. The clinical observations of Head and Riddoch (1917) established the fact that in men with injuries to the spinal cord, there is an exaggeration of the flexor reflexes and that this exaggeration disappears as soon as the patient recovers in cases where injuries have not caused permanent damages to the nervous tissue. This is just what we observed in our frog larvae with the spinal cord severed at different levels. The work of Sherrington (1915) on decerebrate rigidity proved that the transection of the brain at or adjacent to the caudal border of the mesencephalon induces an overreaction of the antigravity muscles. This also is just what we found in our decerebrated tadpoles. The investigations of Magnus (1924) demonstrated that the maintenance of normal body posture and normal orientation to the earth's gravitation field depends on the structural integrity of the midbrain. This, too, is just what we found in our frog tadpoles with the CNS transected at the rostral border of the mesencephalon.

However, the experiments on the larval brain and spinal cord, in comparison with those on the adult nervous system of the frog, show that the

lower centres of the growing CNS are more independent from the higher ones. In adult frogs, the transection of the CNS at the cervical region of the spinal cord, or at the rostral border of the hindbrain, or at the rostral boundary of the mesencephalon leaves them quite inactive, hardly showing any spontaneous activity. But the spinal, the decerebrated and the mesencephalic tadpoles are as spontaneously active as the thalamic and the normal ones (Wang and Lu, 1940). The higher centres seem gradually to acquire an ascendancy over the spinal ones, finally having them entirely under control. The discovery and analysis of this process is an important problem in neurophysiology which still awaits experimental study.

SUMMARY

1. The development of aquatic locomotion in *Rana güntheri* passes through the following stages: (i) the non-motile stage, (ii) the flexure stage, (iii) the stage of the 'S' reaction, (iv) the stage of translatory movements of the body, (v) the stage of the control of the direction of swimming, and (vi) the stage of the maintenance of normal orientation to the earth's gravitation field during motion and rest.

2. The last two stages are easily affected by transverse lesions made in the embryo on the central nervous system. The severance of the spinal cord arrests the development of swimming at the stage (iv); and after the transection of the brain at the caudal border of the mesencephalon, the development of aquatic locomotion does not reach beyond the stage (v). The removal of the forebrain and the interbrain, or of the telencephalon alone has no effect whatsoever on the ontogeny of swimming.

3. The righting reflexes appear in the stage (vi) of the development of swimming; and the transection of the central nervous system at any level caudad to the rostral border of the hindbrain prevents the operated larvae from acquiring these postural reflexes. With tadpoles of 6 mm. body length which have not yet developed the righting reflexes, the removal of both labyrinths produced its usual effect on locomotion in making them lose the control of the direction of swimming.

4. The transection of the spinal cord induces in the larvae an exaggeration of the flexor reflexes of the tail, and consequently causes it to be permanently ventroflexed; while decerebration produces an accentuation of the extensor reflexes of the tail, and consequently makes it permanently extended. Such effects are absent after the transection of the central nervous system rostrad to the rostral border of the midbrain.

5. The normal and mesencephalic larvae can recover from the effects of double labyrinthectomy, if there is no concomitant injury to the hindbrain. On the transection of the brain at the caudal boundary of the mesencephalon immediately return the symptoms of the extirpation of both labyrinths. The decerebrated tadpoles do not recover from the effects of double labyrinthectomy.

6. The spinal, decerebrated and mesencephalic tadpoles are as spon-

taneously active as the diencephalic and normal ones. This is entirely contrary to the findings on the adult frogs.

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THERMOCOAGULATION OF MOTOR CORTEX EXCLUSIVE OF ITS SIXTH LAYER*

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DESTRUCTION of all layers of a circumscribed cortical area by thermocoagulation (at 80°C for 5 sec) completely abolishes the electrocorticogram (ECG) in the entire region of thermocoagulation. If one of the two electrodes is placed 0.5 mm outside the boundary of coagulation a normal ECG is obtained. These two findings demonstrate first, the ECG originates in the cortex, and second, its source is highly localized. After partial thermocoagulation (T C) of a cortical area, the surviving deeper layers, even in the acute experiment, exhibit electrical activity. This activity, however, is not undisturbed. In such an acute experiment the destroyed superficial laminae are still present, preventing the direct lead-off from the layers underneath, furthermore, the coagulation products are acid in reaction, and their diffusion into immediately subjacent cortical layers reduces the electrical activity of the latter.⁵

In the *chronic* T C experiment, on the other hand, the superficial, killed cell-layers have been resorbed completely,² and it is thus possible to obtain from such a "reduced" cortex a true picture of the electrical potentials of the remaining layers. Elimination of the external laminae of the "motor" cortex, in the chronic thermocoagulation experiment, with preservation of the large and giant pyramidal cells as well as those of the polymorphic variety,⁶ results in no essential change of the ECG (Fig 1).

It remained, therefore, to investigate the electrical function of the "motor" cortex reduced to its innermost, or polymorphic layer. In the solution of this problem the following chronic experiments were undertaken.

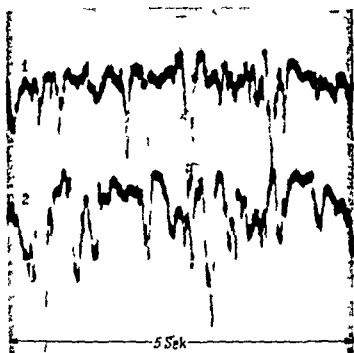


FIG 1. Electrocorticograms from A 4 region of a macaque's brain (Dial narcosis). Record 1 from an area reduced to two inner layers by local thermocoagulation (chronic experiment, 70°C—3 sec), record 2 from normal A 4. No essential difference between the two ECGs. Same amplification in the two records (After Dusser de Barenne and McCulloch).

* The data in this paper are taken from the thesis presented by J P Murphy in partial fulfillment of the requirements for the degree of Doctor of Medicine, Yale University, 1939.

EXPERIMENTS

Experiment 1.—Thermocoagulation of motor cortex with destruction of all but polymorphic ganglion-cell layer. Persistent but eventually remitting paresis of contralateral upper extremity. Marked reduction in electrical activity of coagulated area two months later.

On November 9, 1938, under ether narcosis, the right precentral and post central motor cortex of *Macaca mulatta* was exposed. The entire precentral arm region was thermocoagulated at 75°C. for 6 sec. The procedure was finished in 1 hr. and 45 min. While the animal was still on the operating table and asleep, marked diminution in muscle tone was noted in the left upper extremity. When seen in his cage 2 hr. and 30 min. later the monkey was alert and feeding. Severe paresis was evident in the arm, particularly in its distal portion. The hand was used little, and not at all for finer movements. It was often placed in abnormal positions on the floor. The grip was extremely weak and movements of defense were executed with definite ataxia. A slight improvement in the motility of the paretic limb was noted on the first post-operative day; this became more definite one day later. At the end of the first week gross movements involving the proximal joints were being undertaken.

Two months were allowed to elapse before electrical records were taken. There was now no difference between the two arms in motion and muscle-tone. On January 9, 1939, the animal received 0.5 cc. of Dial (Ciba) per kg. body-weight, half intraperitoneally and half intramuscularly, and was re-explored. The cerebral dura, although not thickened, was adherent to the region of thermocoagulation. The pachymeninx was not removed in order that the electrical activity of the two symmetrical A.4 areas could be recorded under similar conditions. Bipolar stigmatic Ag-AgCl electrodes were placed 2.5 mm. apart on the surfaces of the areas under investigation, and electrical records from the cortex taken with a Westinghouse oscillograph (Fig. 2).

After the taking of ECGs, the position of the two electrodes on the thermocoagulated site was marked by means of a narrow, shallow incision in the dura. The animal was then killed with an overdose of ether. The brain was

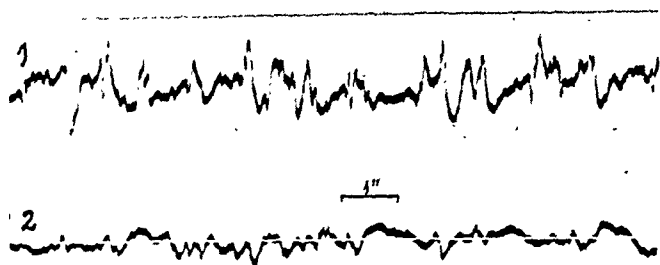


FIG. 2. Electrocorticogram from A.4 region of a macaque's brain (Dial-narcosis) taken through dura. Record 1 is taken from left A.4 (normal), record 2 from right A.4 reduced to inner layer of polymorphic cells (chronic thermocoagulation experiment, 75°C—5 sec.) Same amplification in the two records. Speed of paper: 10 cm. = 7 sec.



FIG. 3. A demonstrative section of the area under the electrodes. Beneath the dura lies a narrow band of fibroblasts and glia. Immediately underneath this may be seen undamaged polymorphic ganglion cells. The large and giant pyramidal cells of the fifth layer are gone.

removed and placed in 95 per cent alcohol for microscopic serial sectioning and staining by means of the Nissl technique.

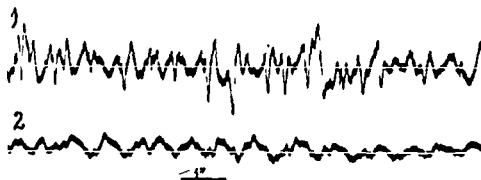


FIG. 4. Electrocorticograms from A.4 regions of a macaque's brain (Dial-narcosis), taken *directly from cortex*. Record 1 taken from left A.4 (normal), record 2 from right A.4 reduced to inner layer of polymorphic cells (chronic thermocoagulation experiment, 71°C—7 sec.). Same amplification in the two records.

Experiment 2.—Thermocoagulation of area A.4 sparing polymorphic ganglion-cell lamina alone. Long-lasting motor deficit in arm of opposite side. ECG recorded from operated site after interval of two months reveals extremely low frequency and amplitude of potentials, as in Experiment 1.

The second animal was operated upon on November 14, 1938. The right sensorimotor cortex was exposed under ether narcosis and laminar thermocoagulation of A.4 was effected at 71°C. for 7 sec. The dura and scalp were closed in the usual manner. Six hours later failure to use the left arm or hand

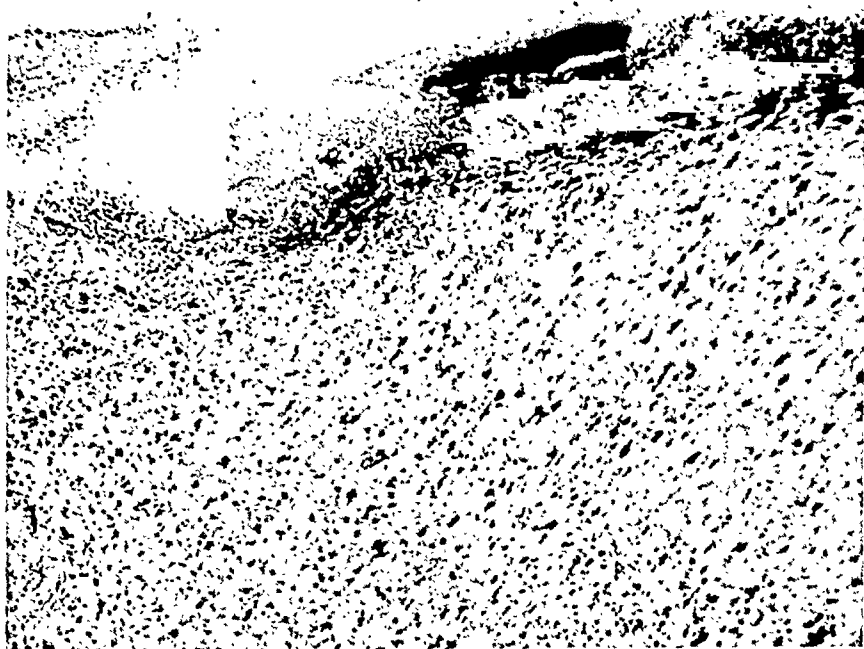


FIG. 5. A representative section of the area underlying the electrodes. On the surface of the resorbed cortex lies a layer of clotted blood, immediately beneath there is fibroblast proliferation together with gliosis. No cells of the large or giant pyramidal types are visible. Below the glia remain intact cells of the sixth, or polymorphic layer.

for picking up food or in defense movements was observed. The extremity was employed in an awkward fashion, however, in walking and climbing. The fingers, hand, and arm frequently remained in abnormal positions. It was possible to abduct the limb far laterally without any correction of posture and without appreciable resistance. The hand was often placed on the dorsum when used for support. The next day, food was being taken only with the right hand. No grasping power could be detected in the left hand when a bar was placed in the palm, but there were gross movements in the proximal joints of the arm. Motor impairment had cleared markedly two weeks later.

After a two-month interval, at the end of which there was no observable

difference between the two upper extremities, the animal received Dial as outlined in the first experiment, and the field of previous operation was re-exposed. The dura could be removed with ease and without damage to the area of thermocoagulation. The left sensorimotor arm region was uncovered and stripped of its dura as well. Electrodes were placed at a distance of 2.5 mm from each other on the surfaces of homologous gyri, and electrical activity was then recorded (Fig. 4).

Following the marking of the position of the two electrodes on the thermocoagulated cortex by means of a very fine linear incision, the animal was killed with ether, and the brain was fixed in 95 per cent alcohol for serial sectioning and Nissl staining (Fig. 5).

All of the sections from the region of coagulation were carefully studied. In some few of the sections the resorption of the destroyed cortex included polymorphic cells, their place being taken by glia. However, these "glial clefts" were narrower than the diameter of the electrode-bulb.

DISCUSSION

It is evident from a consideration of Fig. 1 as contrasted with Fig. 2 and 4 that there is a marked dissimilarity between the electrocorticogram obtained from the "motor" cortex reduced to its two inner layers, and that taken from a similar cortical region in which nothing but the last, or polymorphic cell-layer remains. In the former, the ECG is indistinguishable from that of the normal area 4—in the latter, there is manifest a striking reduction in frequency and amplitude of electrical potentials. The difference between the two sets of experiments is only attributable to the fact that in the latter the layer of large and giant pyramidal cells has been eliminated.

Another salient point of contrast is that while the animal whose "motor" cortex has been destroyed only to the extent of the four outer layers exhibits at most a very slight and transitory motor impairment,¹ that in which thermocoagulation of the "motor" area has included the layer of large and giant pyramidal cells evidences typical and long-lasting motor deficit, as may be seen in the protocols outlined above.

CONCLUSIONS

Significant diminution of the electrocorticogram and pronounced, long-lasting motor deficit are produced by laminar thermocoagulation of the precentral "motor" cortex, when the coagulation is extensive enough to include the large and giant pyramidal cells, but not layer VI, in area 4 and the homologous fifth layer in the other precentral arm areas (A 4-s and A 6).

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TRANSMISSION OF IMPULSES THROUGH THE BURDACH NUCLEUS

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THE SEQUENCE of potential changes that takes place after the stimulation of dorsal root fibers, as recorded with electrodes placed on the segment of the dorsum of the spinal cord corresponding to the root level, is characterized by a primary spike, followed by a slow negative and a positive intermediary potential (Gasser and Graham, 1933). This potential sequence, steadily decreasing in size, can be recorded several centimeters above the level of entrance of the afferent volley (Hughes and Gasser, 1934a), and with needle electrodes in the proper tract of the dorsal column the primary spike can be followed up to the medulla (Hursh, 1940). The purpose of the present investigation has been to follow the transition of the impulses from the primary neurons to the secondary neurons of the nuclei of Goll and Burdach. For the sake of convenience the afferent fiber system connected with the Burdach nucleus has been used more or less exclusively, but supplementary information has been obtained from the Goll system as well.

A few anatomical facts of interest may be recalled in this connection. According to Cajal (1909) collaterals of the long ascending branches of the dorsal root fibers are given off in the greatest number to the grey matter of the spinal cord over three or four segments above the root, and then to the nuclei of Goll and Burdach in the medulla. In the latter the connections are made with the cells that form the arcuate fibers, without the intervention of interneurons within the nuclei. Thus a straight relay occurs in sufficient anatomical isolation from the adjacent complex synaptic systems to provide a favorable situation in which relays of this sort may be studied physiologically without undue interference arising from simultaneous activity in the neighborhood. The dorsal root fibers from the cervical and upper thoracic segments terminate in the cuneate (Burdach) nucleus and partly in the external cuneate nucleus of Clarke. Monakow (Ferraro and Barrera, 1935), while fibers from the lumbar and sacral segments terminate in the gracilis (Goll) nucleus. The synaptically transmitted impulses of the Goll and Burdach nuclei cross the midline in the internal arcuate fibers and pass to the thalamic nuclei in the medial lemniscus.

METHODS

Cats decerebrated or under Dial narcosis (0.5 cc/kg) were used exclusively. The spinal cord was usually exposed down to the lower cervical segments. The posterior part of the skull was opened and the cerebellum removed, thus exposing the floor of the IVth ventricle as well as the anterior part of the Goll and Burdach nuclei. For stimulation, the ulnar, median and radial nerves of the forelimb were used either separately or in combination. In the hindleg stimulating electrodes were applied to the sciatic nerve trunk. To record the potential changes in the spinal cord and the medulla a small Ag-AgCl ball electrode was placed on the dorsal surface, with an indifferent electrode on inactive tissue. For studying

electrical activities within the nervous structures a steel needle having a tip diameter of approximately 50μ and insulated to its tip was used. The customary stimulating device and differential amplifier system were employed.

The rectal temperature of the cats was kept between 38° and 39.5°C . In several of the cases the exposed part of the central nervous system was covered with oil.

RESULTS

The characteristic potential changes described by Gasser and Graham (1933) as obtained in the lumbar region after a dorsal root stimulation are also produced in the lowest cervical segments when a stimulating shock is ap-

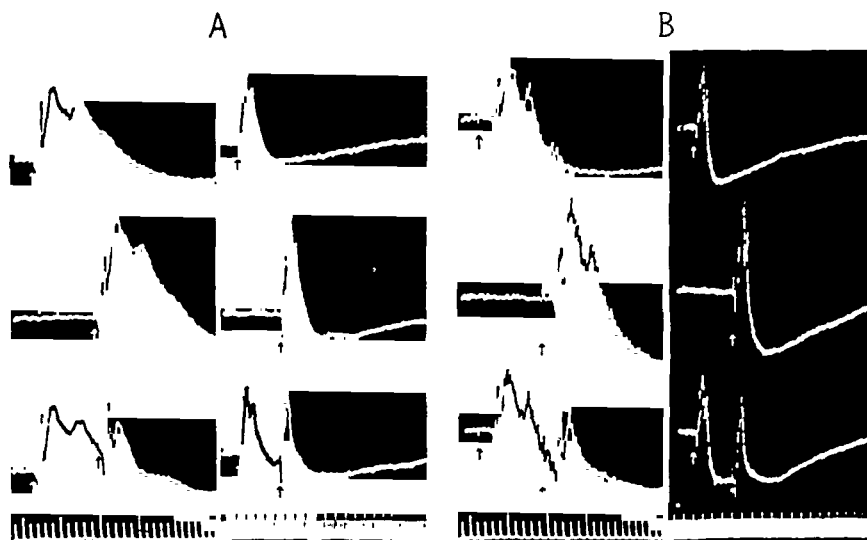


FIG. 1. Two stimulating shocks applied to the ulnar nerve; the first shock of sub-maximal strength, the second supramaximal. Records taken with surface lead, in column A at C₇, in column B at the cuneate nucleus. Shock intervals 9 and 20 msec. Shock artifacts marked with arrows. Time, 1 and 5 msec. Cat under Dial narcosis. Upward deflection in these and all subsequent records indicates negativity at the "active" lead.

plied to the ulnar, median, or radial nerve of the forelimb. Figure 1A shows these familiar features, with the primary spike followed by a prolonged negative wave, on the descending phase of which a second negative elevation is commonly seen. This elevation corresponds to the "first negative complex" of Hughes and Gasser (1934a). The duration of the negative and positive waves respectively, as well as the way in which they are modified by a preceding volley, is also in correspondence with the generally known facts about the potentials in the lumbar part of the spinal cord (Hughes and Gasser, 1934b). As the primary volley proceeds towards the cuneate nucleus, the same potential sequence, though decreased in size, is reproduced in the higher segments. When the nucleus itself is reached, the form of the action potential still follows the same general contour (Fig. 1B) but some differences appear. All the potentials are larger, particularly the positive potential, and the negative potential following the primary spike is shorter.

The primary spike. The usual distance of conduction from the stimulated point of the forelimb nerve to the nucleus was 9–10 cm., necessitating a conduction time of 1.5–2.0 msec. Shocks to the nerve strong enough to stimulate all the alpha fibers produced maximal tract spikes; and as the duration of the spike at the medullary level was only about 1.0 msec., the indication is that a fairly homogeneous set of fibers is involved.

The post-primary negative potential at the cuneate nucleus. Measurements of the duration of the post-primary negative wave are made inexact by the shifting of the base line occasioned by the concomitantly recorded positive potential. But 5 msec. may be taken as a fair average value. About 3 msec. after the arrival of the primary spike a second negative elevation is commonly recorded on the falling slope of the first elevation.

In connection with the negative potential following the primary spike, the question must be raised as to whether all the potential is generated in the nucleus in sequence to the impulses that are recorded in the primary spike, or whether a part of it may be set up by impulses already relayed through cord neurons, and recorded, therefore, as a part of the post-primary cord potential. Against the latter idea is the consideration that, while there are known to be numerous intrinsic fibers in the dorsal columns of the spinal cord (Tower, 1937), none of them is known to make connection with the nucleus. Evidence against the idea is also obtained in a physiological experiment. If the relaying of impulses at any segment of the cord is blocked, thereby eliminating the local production of potentials, a lead from the segment will reveal only the potentials in the fibers that are passing by the segment in the tracts. Blocking can be accomplished by injecting small amounts of distilled water, 0.1 to 0.2 cc., into the grey matter beneath the dorsal column. Figure 2 shows the potential changes recorded at levels 1 cm. apart at approximately the following cervical segments: C₆–C₇ (record 1), C₅ (2), C₃–C₄ (3), C₂ (4), C₁ (5), and at the cuneate nucleus (6). An injection was made into the grey matter at the level of C₃–C₄. As a result it is seen that, while at all other levels the negative and positive post-primary potentials retain their normal form, at the affected segment the potential is limited to the primary spike and a small negative potential separated from it by 2–3 msec. (record 3). From this result it follows that the potentials segmentally recorded are, outside of the exception indicated, locally produced and that they do not represent relayed activity passing toward anterior parts of the nervous system.

The second exception can be identified both by the time lag behind the primary spike and in other ways with the dorsal column relay (Hursh, 1940), which is associated with the dorsal root reflex described by Toennies (1938).

While evidence is lacking for the intervention of relayed impulses other than the dorsal column relay of Hursh, in the setting up of the post-primary negative potential of the nucleus, the idea cannot be dismissed unless it can be shown that the duration of the potential can otherwise be accounted for. In all, a duration of 5 msec. must be explained. The arrival of the dorsal root relay about 3 msec. after the start of the primary spike could account for the

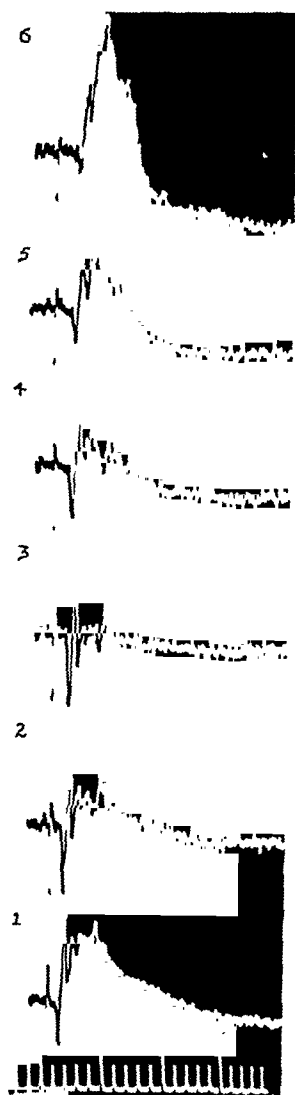


FIG. 2. Stimulation of ulnar nerve. Records taken with surface lead at each of the following levels which were 1 cm. apart: 1 at C_6-C_7 ; 2 at C_5 ; 3 at C_4-C_5 ; 4 at C_3 ; 5 at C_2 ; 6 at the cuneate nucleus. An injection of 0.2 cc. of distilled water into the grey matter was made previous to records at level of record 3. Note absence of intermediary potentials. The primary spike and the dorsal column relay persist at the level of injection. Shock artifact visible in all records. Time, 1 and 5 msec. Cat under Dial narcosis.

production of the later part of the potential. Hence it is upon the first part of the potential that attention must be focused. Only two times can be brought into the argument: the synaptic delay and the duration of the potential in active secondary neurons. The minimal synaptic delay at the cuneate nucleus was found to have a constant value of 0.6 msec., which is in good agreement with synaptic delays elsewhere (Lorente de N6, 1935 and 1938; Renshaw, 1940). If in addition there are longer delays, they cannot be measured, as the start of the relayed potential would be obscured by the potentials set up after the minimal interval. However, it might be safe to assume a variation equivalent to that seen in other situations (Lorente de N6, 1938; Renshaw, 1940), in which case 1.0 msec. could be taken as the maximum.

About the duration of the potential in the nerve cells there is less certainty, because of the difficulty of interpreting the form of the potentials led from active tissue surrounded by a conducting medium. Measurement of the potential recorded by Lorente de N6 (1939) when an antidromic volley was fired into a pool of motoneurons indicates a duration of 1 msec.; and that 1 msec. may be taken as a fair

figure for the neurons of the cuneate nucleus is indicated in some of the records published in the present paper. For instance, the duration of the activity in a highly synchronized group of neurons recorded in Fig. 8 is about 1 msec. Earlier in the paper the duration of the primary spike as recorded at the nuclear level was given as 1.0 msec.; but again this value is only approximate, because of the triphasic character of the record.

If we consider the primary volley coming to the Burdach nucleus to last 1.0 msec. and the maximal synaptic delay to be 1.0 msec., then the last of the potentials in the secondary neurons would start 1.5 msec. after the beginning of the primary volley. If to this time we add 1 msec. for the duration of the potential of the secondary neurons, the total is 2.5 msec., which is slightly

under the 3 msec. period, the filling of which the problem demanded. On the assumption that the missing 0.5 msec. could be made up from the uncertainties in the basic data, it would be possible to account for the post-primary negative potential in the Burdach nucleus on the basis of a two-neuron chain aided by the dorsal column relay; but the reservations about the adequacy of the accounting are so serious that the question of whether there may be some, as yet unidentified, relayed impulses coming into the nucleus should be left open.

Activity in the medial lemniscus. At practically the same time at which the post-primary negative potential starts in the nucleus (0.6 msec.), the first impulses can be recorded with a needle electrode in the medial lemniscus. In obtaining the action potential from the medial lemniscus fibers it has been found convenient to injure the fibers at the point of recording by pushing the needle through the bundle and then withdrawing it for about 1 mm. Thereby the small triphasic deflections that would be obtained from intact fibers are converted into large positive deflections. The form of the potential as led in this way is shown in Fig. 3. Listed in order, beginning at the top, the figure shows records obtained at the following levels: C_2 , C_1 , cuneate nucleus, medial lemniscus after decussation, and medial lemniscus 1 cm. cephalad.

Above the nucleus, as would be expected, the primary spike is no longer visible. By measuring from the shock artifact and allowing 0.17 msec. for conduction, it can be shown that the discharge into the lemniscus fibers starts at the beginning of the post-primary negative potential in the nucleus. The discharge lasts as long as the nuclear negativity persists. (In the figure the duration of the latter is not easily determined because of the displacement occasioned by the positive potential.) It will also be noted that 3 msec. after the start of the lemniscus potential there is a discrete wave marking the appearance of the discharge from the nucleus set up by the dorsal column relay. In the lemniscus there is no activity correlating with the positive potential recorded at the cuneate nucleus.

The positive potential. The activity that supplies the large positive deflection in surface leads from the nucleus appears for the most part not to be directly connected with the nucleus itself. Support for this statement is found in the fact that the negative and positive deflections can be dissociated, and also in the findings obtained with an exploring needle.

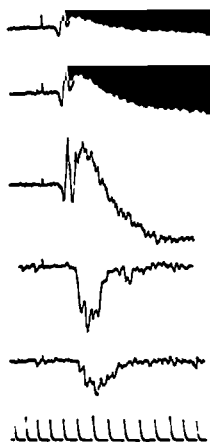


FIG 3 Stimulation of ulnar nerve. Conduction distance between each record 1 cm. Beginning from above the records are taken at the following levels: C_2 , C_1 , cuneate nucleus, medial lemniscus, and medial lemniscus 1 cm further cephalad. Note positive deflections in these and subsequent medial lemniscus records due to recording from "killed ends" Time, 1 and 5 msec. Cat under Dial narcosis

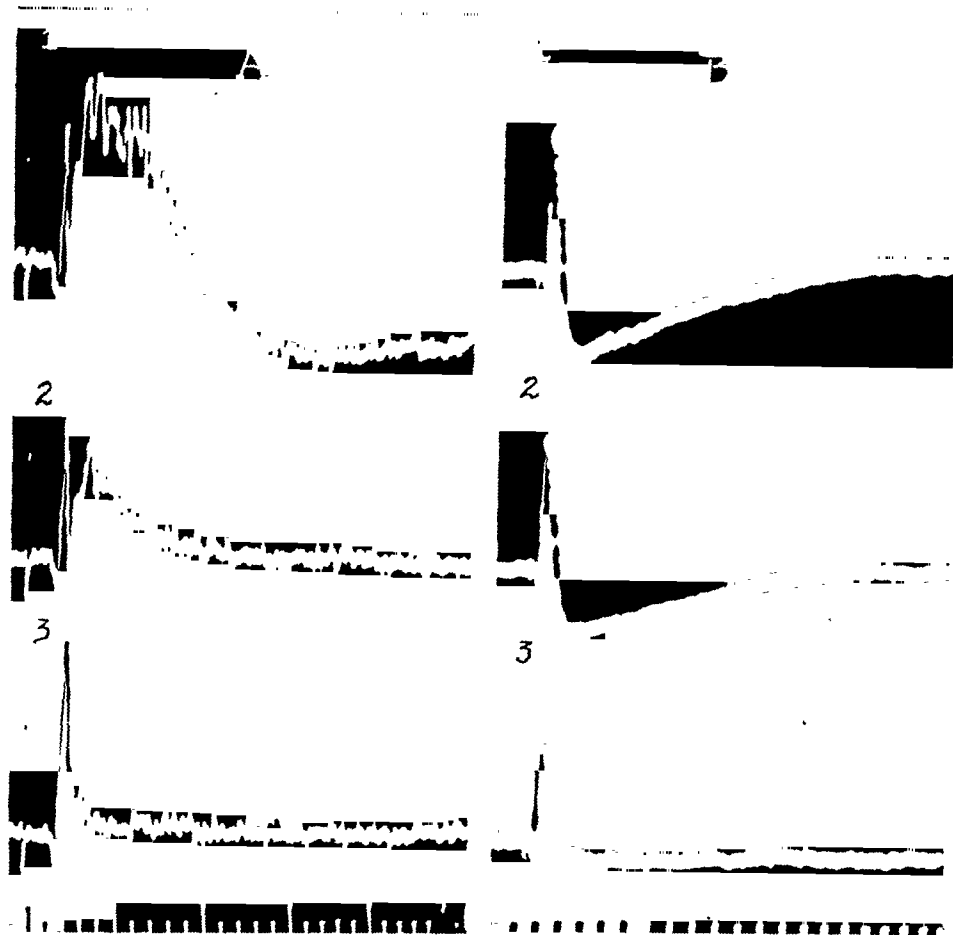


FIG. 4. Stimulation of median nerve and recording with surface lead at the cuneate nucleus, *A* and *B*, from two different experiments. Uppermost records (*A*₁ and *B*₁) taken with cats in normal conditions. In column *A* records taken at different stages of circulatory asphyxia after heart was stabbed. In column *B* the records 2 and 3 were taken at two early stages of progressive respiratory asphyxia. Time in *A*, 1 and 5 msec.; in *B*, 5 msec. Cats under Dial narcosis.

Injection of distilled water at a level 3–4 mm. below the surface of the medulla may reduce the size of the positive potential, without affecting that of the negative potential. Experimentally, however, it was found impossible to obliterate the positive potential entirely without affecting the negative potential.

Dissociation of the negative and positive components can also be effected by asphyxia, to which the positive component is the more susceptible (Fig. 4). The records in column *A* are from an experiment in which the heart was stabbed, but they are also typical for experiments in which respiratory asphyxia was produced. Record 1 was taken before the circulation was stopped, and 2 and 3 during successive stages of asphyxia. In 2 the positive

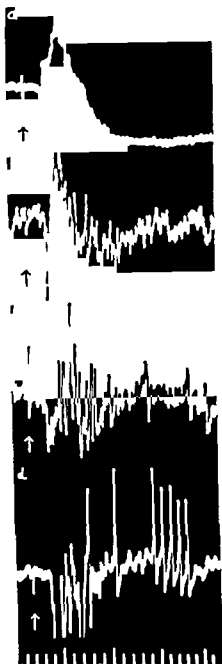
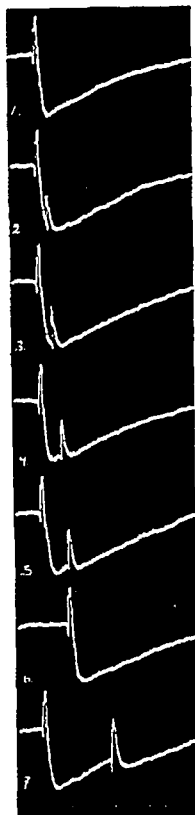


FIG 5 Stimulation of median nerve. Records obtained with a needle electrode *a* on the surface of the cuneate nucleus, *b*, *c*, and *d*, at depths of 0.5, 1.0 and 2.0 mm in the nuclear region. Amplification for records *b*, *c*, and *d* approximately the same as for *a*. Time, 1 and 5 msec. Cat under Dial narcosis.



FIG 6 Same experiment as in Fig 5 but surface record (upper) obtained with ordinary surface lead, the lower record was taken with the needle in new position at a depth of 2+ mm. Time, 5 msec.

FIG 7 Stimulation of ulnar nerve with two maximal shocks (6 times threshold) at different shock intervals. Recording with surface lead at the cuneate nucleus. Record 1 control of conditioning volley, 6 control of testing volley. Note completed recovery of negative post primary potential in record 4 at a shock interval of 15 msec. Time 5 msec. Cat under Dial narcosis (0.4 cc per kg).



wave has disappeared, but the negative wave has also decreased in size. At a late stage of the asphyxia (3) only the primary spike remains. In column B it is shown that the positive wave may almost completely disappear before any change in the size of the negative wave has taken place. The effect is reversible, and full recovery was obtained during the following period of normal breathing.

The findings obtained with an exploring needle are illustrated in Fig. 5, which shows leads obtained at three different depths in comparison with a surface lead. The first change observed as the needle penetrates the pia is the breaking down of the negative component into spikes (b). As the needle is pushed further, the spikes become more prominent and all signs of a positive wave are lost (c). When the needle goes beneath the nucleus at the depth of 2 mm., the spikes are recorded with their phases reversed and a late series of large spikes appears (d). The latter are also recorded at deeper levels. This prolonged discharge evoked by a single afferent volley, which may last for 50 msec. or more, is correlatable with the time course of the positive wave on the surface (Fig. 6). There can be little doubt that it is the activation of the reticular substance in this region that gives the prolonged discharge. This interpretation is supported by the facts that the reticular formation appears at a depth of about 1.5–2 mm. from the surface (Winkler and Potter), and that similar activities in corresponding regions are recorded after an afferent sciatic volley.

In view of the foregoing sets of evidence the positive potential recorded from the surface of the nucleus must be largely attributed to activity outside of the nucleus, and to this extent it is an artifact as far as the nucleus is concerned. As such it would obscure any positivity developed intrinsically in the nucleus; consequently it should not be assumed that no positivity of this sort is developed in the nucleus.

Response to two stimuli applied to the same nerve. The effect of a conditioning volley on the spinal cord potentials is well known from previous work (Gasser and Graham, 1933; Hughes and Gasser, 1934b; Hughes *et al.*, 1937). That an obvious similarity exists between the reaction of the cuneate nucleus and the spinal cord is shown in Fig. 1.

The recovery of the negative potential at the cuneate nucleus has been determined in a series of experiments using two supramaximal shocks, 6 to 7 times threshold. In several cases it has been found that the negative wave has regained its full size at shock intervals of not more than 15 to 20 msec. (Fig. 7). But there is considerable variation from preparation to preparation as to the time required for full recovery. It is often necessary to increase the shock interval to 50 msec. or even more, until the negative wave has recovered completely. The cause of this variability has remained obscure, but it has not been possible to correlate it with the size of the positive wave.

The effects of a conditioning volley at short intervals before the testing volley are shown in Fig. 8. When the interval is shorter than 2 msec. the properties of the primary axons become a determining factor (Gasser and Graham, 1933). Thus at an interval of 1 msec. (see figure) the conduction time of the second primary spike is prolonged and the spike is reduced in size. At this interval the second primary spike still, however, clearly elicits an immediately following short-lasting negative wave, which is found superimposed on the negative wave produced by the conditioning volley. Although reduced in size, a negative wave of this sort can be found at any shock in-

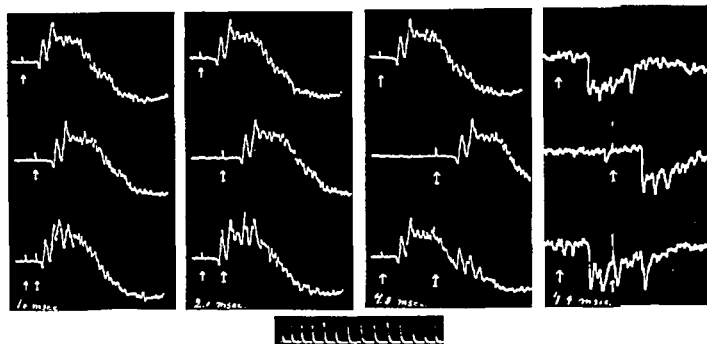


FIG 8 Stimulation of ulnar nerve with two maximal shocks (7 times threshold) Recording with surface lead at the cuneate nucleus at shock intervals of 1.0, 2.1, and 4.8 msec, and with a needle electrode in the medial lemniscus at a shock interval of 4.9 msec. Shock artifacts marked with arrows. Time, 1 and 5 msec. Decerebrated cat.

interval at which a second primary spike can be evoked. The duration of the wave added by the second shock to the pre-existing negativity is about 1 msec., and it is interesting to note that this duration remains constant until the shock interval is increased to about 5 msec, i.e. the duration of the unconditioned negative wave as determined at the baseline level. The height of the conditioned negative wave never exceeds the height of the corresponding part of the unconditioned response and the time interval between the crests of the primary spike and the negative wave remains at its unconditioned value, i.e. approximately 0.6 msec. This finding is shown in Fig. 8 at shock intervals of 2.1 and 4.8 msec. (It may be noted that at the shock interval of 4.8 msec. the second primary spike is decreased in size, presumably due to occlusion by the dorsal column relay of the conditioning volley.) After 5 msec. the duration of the conditioned response begins to be prolonged, and the subsequent course of the recovery is as has been described above.

The records in the last column of Fig. 8 were ob-

FIG 9 Same experiment as in Fig. 7. Usual surface picture taken at the cuneate nucleus in uppermost record, below, similar recording but tetanic stimulation at a frequency of approximately 150 per sec. The lower most record shows the discharge in the medial lemniscus at the same stimulating frequency. Shock artifact visible only in the medial lemniscus record. Time, 1 and 5 msec.



tained from the medial lemniscus (approximately 1 cm. post-synaptic conduction) at a shock interval of 4.9 msec. When a comparison is made of these records with those taken at a corresponding shock interval from the surface of the cuneate nucleus, it is obvious that the two have similar features. In the medial lemniscus the response to the second shock is also shortened to a duration of about 1 msec. and its size, as compared to the unconditioned response, is somewhat reduced. A similar correspondence between the nuclear and the lemniscus responses occurs at other shock intervals as well, and it is still maintained during a tetanus. In Fig. 9 the two upper records were obtained with a surface lead from the nuclear region, and the lower record with a needle electrode in the medial lemniscus. The frequency of the tetanus in both cases was about 150 per sec. The negative wave pro-

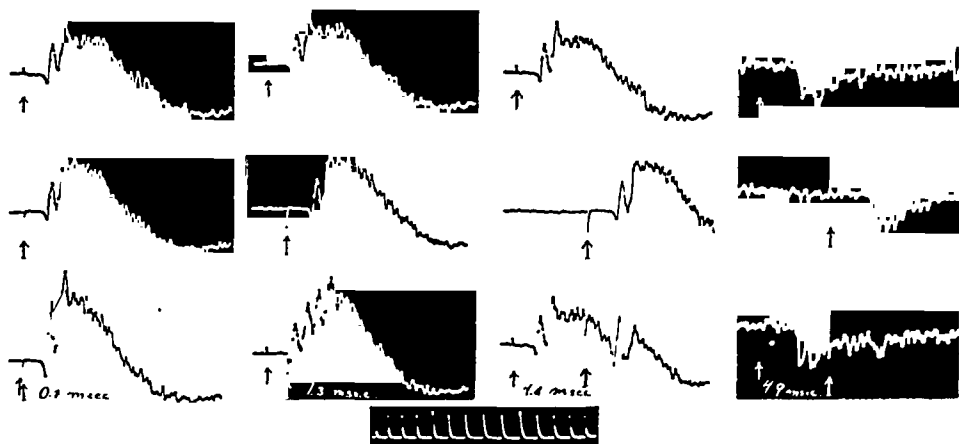


FIG. 10. Same experiment as in Fig. 8. Similar recording and identical amplification as well as speed of sweep. The two shocks delivered to different nerves (ulnar and median nerves) at intervals of 0.1, 1.3, and 4.8 msec., and at 4.9 msec. in the record from the medial lemniscus.

duced by the second shock is somewhat reduced, but it will be seen that a further reduction does not occur during succeeding shocks. The same statement applies to the discharge in the medial lemniscus.

All activity relating to the dorsal column relay, whether it is recorded in the nucleus or in the lemniscus, is inhibited for a long period by a conditioning volley, as would be expected from the description by Hursh of the conditioning of the relay itself.

Response to two stimuli applied to different nerves (Fig. 10). When the two shocks are delivered at approximate simultaneity (0.1 msec. shock interval), a complete summation of the primary spikes is obtained, but the size of the negative post-primary potential recorded at the nucleus is smaller than the sum of the individually obtained responses. Most of the deficit occurs in the late part of the wave, and there is in fact a considerable addition of the potentials appearing during the first millisecond after the primary spike. A

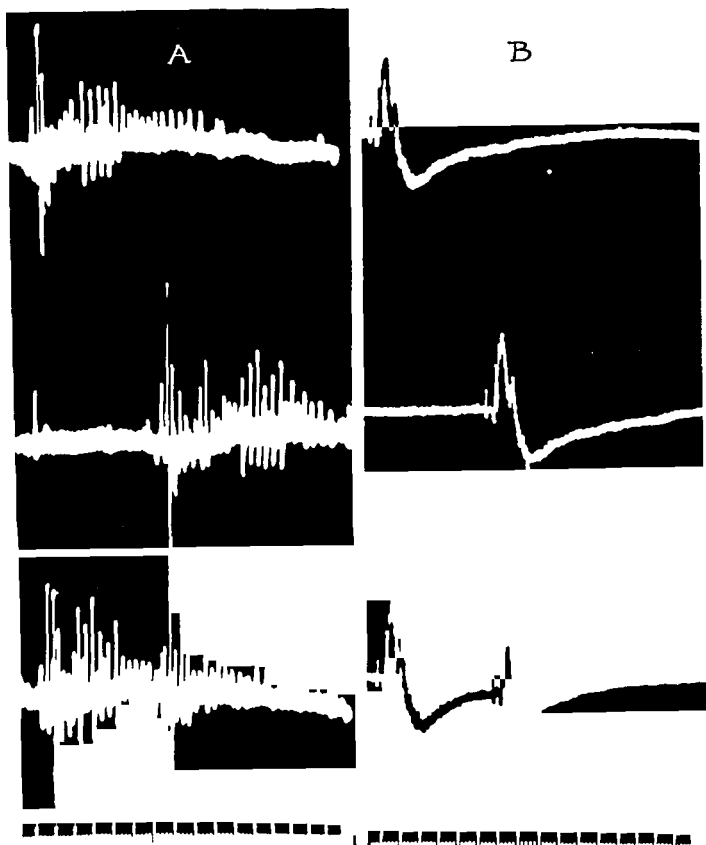


FIG 11 Stimulation of median nerve with two maximal shocks at an interval of 30 msec. In column A the records were taken with a needle electrode inserted to a depth of 2 mm below the surface of the cuneate nucleus. The records show, beginning from above conditioning volley alone, testing volley alone, conditioning volley and testing volley 30 msec apart. Corresponding surface records are in column B. Time, 5 msec. Cat under Dial narcosis.

similar addition occurs when the volleys are separated by short intervals. After 4 to 5 msec the later part of the conditioned negative wave starts to regain its size. Recovery is usually complete at intervals of 15 to 20 msec,



FIG. 12. Stimulation of sciatic and median nerves (ipsilateral). The potential changes are recorded with a surface lead at the cuneate nucleus. Record 1, sciatic volley alone; 2, median volley alone; 3-7, sciatic volley succeeded by the median volley at progressively increasing time intervals. Time, 1 and 5 msec. Cat under Dial narcosis.

but longer intervals, up to 50 msec., may sometimes be required. These effects on the negative wave elicited by a conditioning volley are again reflected in the medial lemniscus response by corresponding changes. It may be noted that neither response attains a supermaximal size, nor is a reduction of the latency demonstrable at any shock interval.

In another series of experiments the first of two shocks was applied to the sciatic nerve and the second to the median nerve of the homolateral forelimb. The usual records were taken with surface leads at the Burdach nucleus, a location which is obviously not favorable for recording any potential changes arising in the Goll nucleus. Figure 12 shows the effect of the sciatic volley alone (1) and succeeded at different intervals by the median volley (3, 4, 5, 6, 7); and the effect of the median volley alone (2). It is obvious that the size of the negative post-primary potential produced by the median volley is little, if at all, affected by a preceding sciatic volley. On the other hand, the positive wave is greatly reduced. This reduction is greatest if the median volley arrives at a time when the positive wave produced by the sciatic volley has reached its maximum.

Positive wave as affected by a conditioning volley. Irrespective of whether the two shocks are applied to the same or to different nerves of the forelimb the amount of positivity that can be added by a second volley at a certain shock interval is practically the same. This increase of positivity becomes appreciable at shock intervals of about 2 msec. Successively longer shock intervals are associated with a progressive increase of the positivity added by the second volley (Fig. 7).

The last spike discharge obtained with a needle electrode at a depth of about 2 mm. below the cuneate nucleus is also affected by a conditioning volley. In Fig. 11, column A, it is seen that the effect of the second volley is reduced in duration. As the occlusion of the positive potential recorded from the surface of the nucleus (column B) parallels the deficit

in the conditioned train of spikes, additional support is given to the interpretation of the positive wave as the potential effect at the surface of the cord of activity in the reticular substance.

DISCUSSION

The outstanding feature of the cuneate nucleus is its powerful one-to-one relay. A volley entering the resting nucleus, after a minimal synapse time of 0.6 msec., produces a burst of activity in the cuneate neurons, which is reenforced about 3.0 msec. later by the arrival of the relayed discharges in the dorsal columns described by Hursh. The total activity lasts about 5 msec. and is recorded by an electrode on the surface of the nucleus as a negative wave. Throughout the continuation of the wave relayed impulses pass anteriorly in the lemniscus fibers.

The powerful part of the relay is confined to the early period of the discharge. Transmission is then so effective that if two shocks are applied to a peripheral nerve at any interval sufficiently long to produce a second response in the nerve fibers, the second volley on arriving at the nucleus will set up a short burst of impulses in the tract (lasting about 1.0 msec.). Up to intervals of 5 msec. the relayed impulses are confined to this short burst, then the latter begins to be followed by other impulses. As the interval is increased, the discharge is prolonged and augmented in its later portions; and resting-control dimensions are attained after 15-50 msec., depending upon the conditions.

From this brief description it is apparent that in addition to direct and powerful relays through the nucleus there are also pathways that are easily conditioned. Part of these pathways can be accounted for through the dorsal column relay. The latter takes fully as long to recover from previous activity as does the discharge in the lemniscus. Hence in so far as the dorsal column relay contributes to the lemniscus discharge, the latter cannot reach full value before the former. All of the easily conditioned pathways cannot be explained in this way, however; because there is a conditioned deficit in the discharge during the first three milliseconds before the dorsal column relay arrives. How one would explain the conditioning depends on how one explains the production of the potential itself during this period. If, contrary to any knowledge about them, one postulates other prenuclear relays, the explanation would be in the pattern obtaining for the dorsal column relay. If, on the other hand, one holds that the activity is all set up by the primary tract spike, then one has to assume that some of the branches of these fibers make less powerful connections than do others, and that a higher degree of recovery is necessary for the weaker connections to have threshold stimulating value than for the stronger.

SUMMARY

Following a single shock applied to a nerve of the forelimb there passes through the dorsal column to the nucleus of Burdach a volley of primary impulses succeeded after about 3 msec. by the dorsal column relay of Hursh. These impulses produce in the nucleus (surface lead) a post-primary negative potential lasting about 5 msec. During the course of this potential, impulses are discharged into the lemniscus.

Conditioned transmission through the nucleus has the following properties: The refractory period at the synapse is not longer than that of the primary axons. Transmission after the second of two volleys at intervals less than 5 msec. is limited to a short burst of spikes (lasting ca. 1.0 msec.) following the second primary volley by a synapse time of 0.6 msec. Addition of the later, more inhibitable, portion of the transmitted impulses starts at intervals of 5 msec. and restoration is complete at 15 to 50 msec. Thus the transmission of a tetanus through the nucleus is essentially one-to-one conduction between primary neurons and neurons of the lemniscus fibers.

A positive wave lasting about 100 msec., recorded at the surface of the nucleus, is correlated with activity in the underlying reticular substance.

I wish to express my sincerest appreciation and thanks to Dr. Herbert S. Gasser for his encouraging interest and criticism during the course of this investigation.

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INFLUENCE OF DISCHARGE OF MOTONEURONS UPON EXCITATION OF NEIGHBORING MOTONEURONS

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INTRODUCTION

SOME spinal motoneurons are equipped with recurrent collaterals which arise from the axon near its origin and terminate in association with other neurons of the ventral horn (cf. Cajal, 1909). An impulse that sweeps over the motor axon must also invade its collaterals. Does it then affect the excitability of the neurons in association with which the collaterals terminate? Müller (1835) could produce no muscular contractions by stimulating the central end of a cut motor root. Others have likewise failed to find evidence that an antidromic volley produces either a centrifugal discharge in motor axons or activity in other nerve tracts (Mislawski, 1895; Bernstein, 1898; Eccles, 1931). In the absence of known excitatory effects it has been suggested that impulses in recurrent collaterals might lead to inhibition of the activity in the neurons to which they pass (Graham Brown, 1914; Gesell, 1940). The collaterals would then be an important part of the mechanism for reciprocal innervation. Forbes and his collaborators (1933) put the suggestion of Graham Brown to a careful experimental test. They found that a contralaterally evoked reflex discharge into the tibial nerve is not conditioned by antidromic volleys arriving at the cord in the motor axons of the peroneal nerve.

The present experiments show that the antidromic activation of certain groups of motoneurons does condition the reflex discharges of other motoneurons. The conditioning effect is often inhibitory. It is then neither preceded by facilitation nor delayed; inhibition is present when the antidromic volley reaches the cord approximately simultaneously with an afferent volley which fires the testing motoneurons directly after a single synaptic delay. The inhibition must then be caused by events occurring during the synaptic delay at the motoneurons—a period of only 0.9 msec. or less (Lorente de Nó, 1938; Renshaw, 1940). The conditioning volley cannot have fired either the tested motoneurons or premotor interneurons in time for the refractoriness (subnormality) which follows activity to mediate the response deficit (cf. Gasser, 1937a, b; Lorente de Nó, 1936).

In a discussion of this phenomenon it would be misleading to focus attention only upon the possible role of recurrent collaterals. It is not necessary to infer from the early onset of inhibition that a specific inhibitory action is produced by the arrival of impulses at the synapses made by the recurrent collaterals with other neurons. As Grundfest (1940) has pointed out, an alternative explanation for findings of this sort is suggested by the fact that

Conditioned transmission through the nucleus has the following properties: The refractory period at the synapse is not longer than that of the primary axons. Transmission after the second of two volleys at intervals less than 5 msec. is limited to a short burst of spikes (lasting ca. 1.0 msec.) following the second primary volley by a synapse time of 0.6 msec. Addition of the later, more inhibitable, portion of the transmitted impulses starts at intervals of 5 msec. and restoration is complete at 15 to 50 msec. Thus the transmission of a tetanus through the nucleus is essentially one-to-one conduction between primary neurons and neurons of the lemniscus fibers.

A positive wave lasting about 100 msec., recorded at the surface of the nucleus, is correlated with activity in the underlying reticular substance.

I wish to express my sincerest appreciation and thanks to Dr. Herbert S. Gasser for his encouraging interest and criticism during the course of this investigation.

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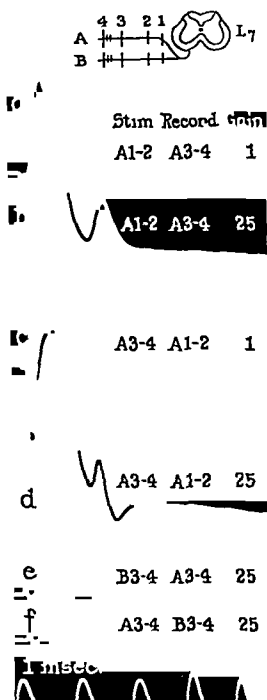


FIG 1 Centrifugal discharges set up in motor axons by antidromic volleys. Cat under light anesthesia (Nembutal). The ventral rootlets of the 7th lumbar segment were divided into two equal groups, as shown in the inset. Relative amplification, stimulating and recording electrodes for each record are indicated on the figure. An antidromic volley in either group of rootlets produced a centrifugal discharge in some fibers of the same rootlets after a central latency of 0.9 msec. Stimulation of either group of rootlets produced no discharge in the other group (records *e* and *f*)

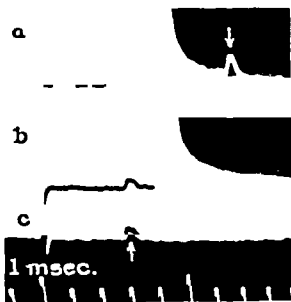


FIG 2 The effect of an afferent volley on the centrifugal discharge which is initiated by an antidromic volley. Records from a small branch of the nerve to the semimembranosus of a cat under light anesthesia (Nembutal). The caudal cord had been transected, and all dorsal roots from the 4th lumbar to the caudal transection were severed on the testing side. The recording leads were placed distal to the stimulating electrodes on the prepared nerve in the thigh *a*, antidromic volley. The deflection due to the centrifugal impulses, as marked by the arrow, appears later than the efferent discharges of Fig 1, because of the greater conduction distance in the motor axons *b*, the antidromic volley preceded by a shock applied to the dorsal columns at L_4 . The efferent discharge following the antidromic volley was almost completely abolished *c*, the small reflex discharge produced by the conditioning stimulus in isolation.

It is not possible to confuse the efferent discharge which follows an antidromic volley with spontaneous firing on the negative after-potential at the cut distal end of the prepared nerve. High partial pressure of carbon dioxide about the distal end greatly reduces the spontaneous firing without altering the centrifugal discharge.

Several additional facts point to a central origin for the discharge which follows an antidromic volley. Not only do the impulses of the discharge pass in a centrifugal direction (Fig. 1*d*), but their presence depends upon the integrity of the spinal cord. The efferent discharge is reversibly abolished during the subtotal asphyxia of the cord, which may be induced by temporary occlusion of the descending aorta at the level of the upper lumbar segments. Lastly, the centrifugal discharge is conditioned by a preceding dorsal root volley which fires few motoneurons (Fig. 2).

While these experiments demonstrate that the centrifugal impulses are set up at the spinal cord, they do not permit a more specific inference about its locus of origin. All that can be said is that the discharge arises at or central to the point of emergence of the motor axons from the cord.

The central latency for the centrifugal discharge evoked by an antidromic volley may be determined from the total latency by subtracting the conduction times in the motor axons for the centripetal and the centrifugal impulses. The total conduction time in the fastest motor axons may be approximated by the sum of the shock-response intervals for *m* waves (Lorente de N6, 1939, page 409) set up by direct electrical stimulation of the motoneurons in the ventral horn and detected at the recording leads and at the stimulating cathode. These corrections for oscillogram *b* of Fig. 1 amount to *ca.* 0.3 msec. The minimal central latency, therefore, is *ca.* 0.9 msec., provided that some of both the centripetal and the centrifugal impulses pass in fibers of maximal or nearly maximal conduction velocity. This assumption is verified by the fact that apparent central latencies calculated in this way from records made on nerves in the thigh are equal to or only slightly greater than the central latencies calculated from the data of ventral root leads.

The central latencies for the centrifugal discharge generally lie between 0.8 and a little over 1.0 msec. The similarity of these values to the synaptic delays at motoneurons (Lorente de N6, 1938; Renshaw, 1940) immediately suggests that the centrifugal impulses may arise from the synaptic excitation of motoneurons by impulses of the antidromic volley arriving over recurrent collaterals. Several lines of evidence render this possibility very unlikely. Instead, the centrifugal discharge seems to be due to repetitive activity in a fraction of the antidromically activated motoneurons. It is, therefore, in some ways formally comparable with the "*effet pseudoréflexe*" of Arvanitaki (1938, page 101; 1940a).

First, the centrifugal discharge never appears in a group of motor fibers other than that in which the antidromic volley passes. This is true when the fibers used for stimulating and recording are groups of ventral rootlets from the same or from adjacent levels of the cord (Fig. 1*e* and *f*). It is also true when mixed nerves of the leg are used (dorsal roots cut), and the same result obtains with motor branches to the same or different muscles.

Second, contributory evidence comes from the relationship between the size of an antidromic volley and the size of the efferent discharge which it

produces. If the efferent discharge were synaptically excited, it would be expected that as the size of the antidromic volley approached its maximal value, the number of centrifugal impulses set up by it would decrease, owing to the large number of refractory motoneurons. Actually a roughly linear relationship exists between the numbers of centripetal and centrifugal impulses. Figure 3 shows some of the responses obtained in one experiment in which the stimulating shock was progressively increased from submaximal strengths to values supermaximal for A fibers.

Additional proof is supplied by the effects which an antidromic volley exerts upon the reflex discharges of other motoneurons. As stated above, an antidromic volley in isolation never sets up centrifugal impulses in other motoneurons. It does, however, condition the reflex discharges into other motor axons. A detailed discussion of these effects follows. In brief, the facilitation which may occur is always delayed; inhibitory effects on the other hand appear immediately upon the arrival of the antidromic impulses into the cord. Therefore, at the time an antidromic volley, which arrives at the cord in some motoneurons, is setting up its centrifugal discharge, it produces only inhibitory effects on the synaptic excitation of other motoneurons. The efferent discharge cannot, therefore, be in motoneurons other than those invaded by the backfired impulses. Detonator facilitation produced by the arrival of impulses at the terminal knobs of the recurrent collaterals has not yet been discovered. One might suppose that such excitation exists; but as far as other motoneurons are concerned, any such action is masked by a more effective inhibitory process acting at the same time.

2. *Effect of antidromic volley in some motor fibers on reflex discharges of other motoneurons.* Antidromic volleys in certain deafferented motor nerves condition subsequent reflex discharges into other motor branches (Fig. 4-8; Table 1). For a given pair of nerves the effect is relatively constant in different preparations. The conditioning is frequently inhibitory; but facilitation, usually preceded by inhibition, also occurs

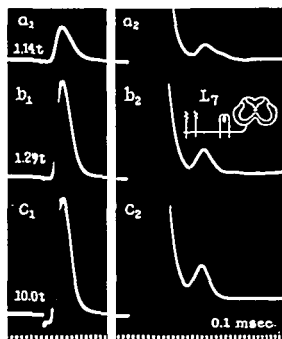


FIG 3 The relation between the size of an antidromic volley and the size of the efferent discharge produced by it. Cat under light anesthesia (Nembutal). Stimulating and recording electrodes arranged as shown in the inset. On the left, low gain records to show the size of the volley directly initiated by the stimulating shock. On the right, records at 25 times the amplification to show the centrifugal discharges of central origin. Stimulus strength, expressed in terms of threshold value (1.0t), is indicated on the left for each pair of records.

An antidromic volley in one group of ventral rootlets often acts to inhibit subsequent testing discharges in the motor axons of adjacent rootlets. In contrast to the results ob-

tained when motor nerves are used for conditioning and testing, the effects are variable and sometimes absent. The reason for the relative constancy of the conditioning effect when motor nerves, rather than groups of ventral rootlets, are used is obvious in the light of cer-

Table 1. Effects of antidromic volleys in some motor nerves on reflex discharges into other motor nerves

	Motoneurons occupied by conditioning volley	Tested motoneurons	Effect of conditioning volley on tested reflex*
Conditioning volley and tested reflex in motor nerves to the same muscle or muscle group	biceps (one part)	biceps (another part)	Inhibition (33, 65)
	biceps (one part)	semitendinosus	Inhibition (50, 64, 75, 70, 75, 20)
	biceps (one part)	biceps (one part)	Inhibition (80, 40, 85, 20)
	semimembranosus	semimembranosus	Inhibition (60)
	semimembranosus (one part)	biceps (one part)	Inhibition (92)
	semimembranosus	semimembranosus (another part)	Inhibition (35)
	medial head of gastrocnemius	semitendinosus	Inhibition (35)
	lateral head of gastrocnemius	lateral head of gastrocnemius	Inhibition (50, 33, 50, 75)
Conditioning volley and tested reflex in nerves whose motoneuron pools lie in different portions of the ventral horn	quadriceps (one half)	medial head of gastrocnemius	Inhibition (60, 60)
	sartorius (one part)	quadriceps (other half)	Inhibition (10, 10, 15, 10, 10)
		sartorius (remainder)	Inhibition (60, 70)
	tibial	peroneal	Facilitation (to 140 percent), preceded by slight inhibition
	peroneal	tibial	No significant effect observed
	tibial	hamstring	Inhibition (61, 85)
	hamstring	tibial	Inhibition (80, 85), followed by facilitation (118, 120)
	peroneal	hamstring	Inhibition (75, 82)
Conditioning volley and tested reflex in motor nerves to individual antagonistic muscles	hamstring	peroneal	Inhibition (85, 92)
	quadriceps	quadriceps	Inhibition (54, 88, 95)
	tibial	hamstring	Facilitation (117)
	quadriceps	quadriceps	Inhibition (76)
	peroneal	tibial	Slight facilitation (?)
	quadriceps	quadriceps	Slight inhibition (?)
	quadriceps	peroneal	No significant effect observed
	sartorius	sartorius	No significant effect observed
	sartorius	quadriceps	Slight inhibition
		gracilis	No significant effect observed
	gastrocnemius	tibialis anticus	Slight facilitation
	tibialis anticus	gastrocnemius	No significant effect observed
	extensor longus digitorum	flexor longus hallucis	No significant effect observed
	flexor longus hallucis	extensor longus digitorum	No significant effect observed

* The figures in parentheses represent the maximal conditioning seen in specific experiments and expressed as:

$$\frac{\text{size of conditioned reflex}}{\text{size of unconditioned reflex}} \times 100.$$

tain anatomical details (Sherrington, 1892, Marinesco, 1904, Cajal, 1909, Bok, 1928) The fibers of any small ventral rootlet are the axis cylinders of motoneurons lying in the cord at approximately the segmental level at which the rootlet makes its exit. They lie scattered throughout the cross-section of the ventral horn at this segmental level (Cajal). The motoneurons associated with any given muscle, on the other hand, extend continuously over two or more segments of the cord, but their cell bodies always occupy a specific, restricted locus in the cross section of the ventral horn (Marinesco, Bok). The motor axons to each muscle thus arise from cells occupying a specific portion of the ventral horn. They become randomly mixed with other motor fibers in the ventral roots, and eventually segregate again into the motor nerve. The segmental variation in the innervation of any particular muscle is considerable, but the neurons representing the various muscles always preserve the same relative positions longitudinally in the cord (Sherrington). Thus in all preparations the motoneurons supplying any muscle stand in the same axial and cross-sectional relationship to those of every other muscle. No such fixed relationship exists between the motoneurons supplying any two groups of rootlets.

Control experiments demonstrate that antidromic conditioning as shown in Fig. 4 to 8 depends upon the arrival at the cord of impulses in motor A fibers. (i) The routine procedure has been to section the dorsal roots at, above, and below the segments which typically receive afferent fibers from the antidromically stimulated nerve. More extensive deafferentation in a few experiments has served to exclude the possibility that the conditioning is mediated by impulses in aberrant sensory fibers. In one such experiment the cord was transected at both caudal and upper lumbar levels. All dorsal roots on both sides of the isolated lumbosacral segments were cut intradurally. Impulses from the periphery could then arrive only via the intact motor roots. A shock applied to the dorsal columns at L4 served to set up a small discharge in a motor branch to the quadriceps. This discharge was inhibited by a preceding antidromic volley in the other branches of the crural nerve, just as when only the homolateral lumbar and sacral dorsal roots had been severed. (ii) The records of Fig. 4 demonstrate that impulses in the A fibers of the conditioning nerve are the ones which produce the inhibition. In the experiment from which the records of Fig. 4 are taken, stimulation of the homolateral dorsal columns at L4 produced a discharge in the nerve to the lateral head of the gastrocnemius (record *a*). In the following records (*b*-*e*) the column stimulus was preceded by a shock applied to the nerve of the medial head of the gastrocnemius. The inhibi-

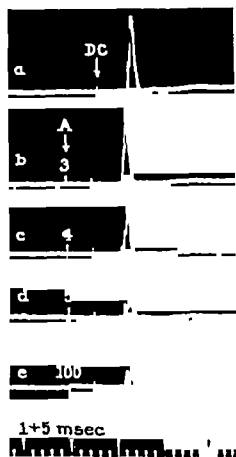


FIG. 4. The conditioning of motor discharges into the nerve to the lateral head of the gastrocnemius by antidromic volleys in the nerve to the medial head of the gastrocnemius. Decerebrated cat. The caudal cord had been transected and all dorsal roots as far cephalad as the second lumbar were severed on the tested side. *a*, motor response evoked by stimulation of the dorsal columns at L4. *b*-*e*, the same preceded by a shock to the nerve of the medial head. Relative stimulus strengths indicated on the figure.

dorsal column at L4 produced a motoneuron discharge which was recorded in the nerve to the lateral head of the homolateral gastrocnemius. This discharge was inhibited by a preceding antidromic volley backfired into the motoneurons supplying the medial head of the gastrocnemius. As shown in Fig. 5, the inhibition reached a maximum when the testing impulses were set up shortly after the antidromic volley. The response deficit then gradually declined and disappeared as the shock interval approached 50 msec. Similar results were obtained from experiments with the various branches to the hamstring muscles, with branches to the quadriceps, and with branches to the sartorius (Table 1).

Of particular interest is the fact that the inhibition of a tested discharge by an antidromic volley in a related group of motoneurons appears when the antidromic volley reaches the cord as late as simultaneously with the testing impulses which fire the motoneurons after a single synaptic delay. The illustrative data of Fig. 6 are taken from an experiment in which the conditioning volley and the tested motor discharge occupied the two principal branches to the quadriceps. The caudal cord was transected and all homolateral dorsal roots as far cephalad as L1 were severed intradurally. The conditioning curve is shown in Fig. 6A. It is clear from Fig. 6B, which shows the data on which the point *x* of 6A is based, that the testing response was definitely inhibited when the conditioning shock preceded the testing stimulus by only 0.7 msec. Oscillograms taken with the stimuli in this temporal relation appear in Fig. 6C. The sequence of potential changes evoked by the conditioning antidromic volley and recorded with a needle electrode within the ventral horn is shown in the upper record. The middle record shows the unconditioned testing response, and the lower record the testing response inhibited by an antidromic volley set up 0.7 msec. before the testing impulses.

More significant than the shock interval is the relationship between the time of arrival of the antidromic volley at the cord and the period of the central latency for the tested motor discharge. Most of the motoneurons of the quadriceps group are located in L5 and L6 (Sherrington, 1892). The upper record of Fig. 6C shows that impulses of the antidromic volley reached the ventral horn of these segments 0.8–0.9 msec. after delivery of the conditioning shock. Direct electrical stimulation of the motoneurons at L5–L6 produced an *m* wave (Lorente de Nó, 1939, p. 409) at the recording electrodes after 0.8 msec. Therefore, 0.8 msec. was approximately the time taken for the tested motor impulses to travel from the cord to the recording electrodes. Thus the second arrows in the lower two records of Fig. 6C indicate the time at which these impulses left the cord. Records from the dorsum of the cord show that the testing impulses arrived at L5–L6, 0.2–0.3 msec. after the testing shock, as indicated by the first arrows on the lower records of Fig. 6C. The central latency of the testing reflex in these segments is given by the interval between the arrows—ca. 0.9 msec., or the duration of a single synaptic delay (Lorente de Nó, 1938; Renshaw, 1940). It is apparent from these conduction times that when the conditioning shock preceded the test-

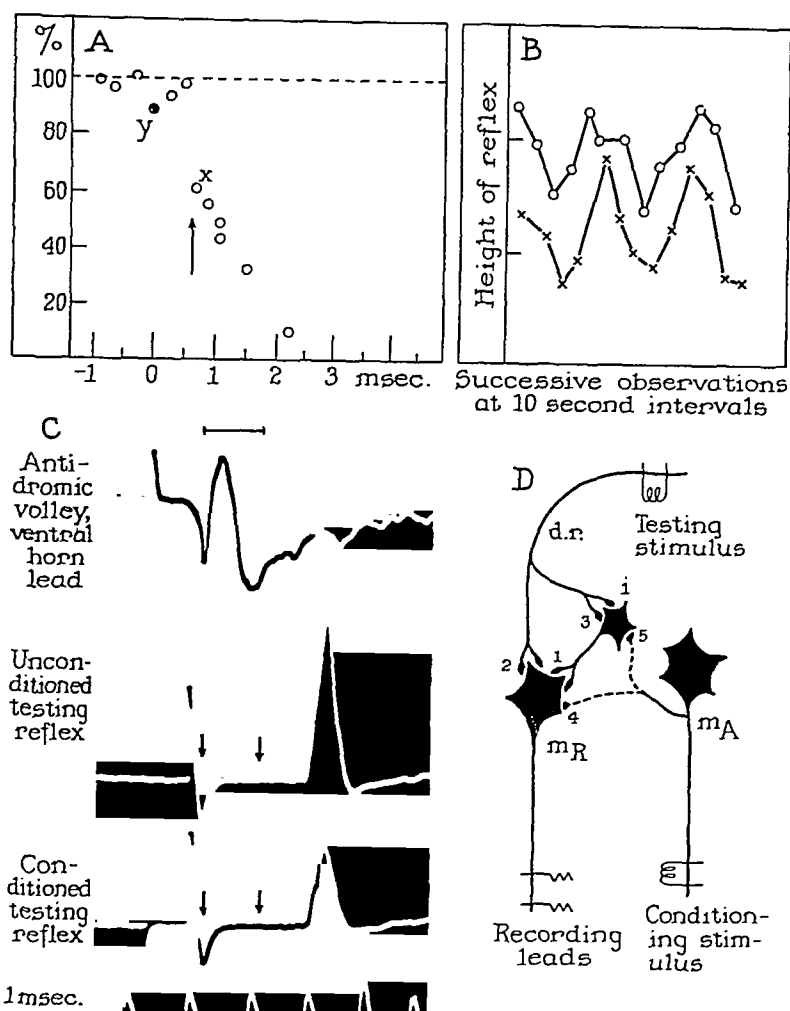


FIG. 6. Conditioning of motor discharges into part of the crural nerve by antidromic volleys in other branches to the quadriceps. Cat under light anesthesia (Nembutal). The caudal cord had been transected, and all sacral and lumbar dorsal roots on the tested side were cut. The tested "reflexes" were initiated by stimulation of the dorsal columns at L4. 6A, the conditioning curve, ordinates and abscissae as in Fig. 5. The arrow indicates the time at which the conditioning and testing volleys reached the ventral horn simultaneously. 6B, the data on which point *x* of 6A is based. Note the definite inhibition superposed upon a slow rhythmic variation of the discharge. 6C, oscillograms identified on the figure. The abscissae are on the same scale as those of 6A. The conditioning and testing shocks have the temporal relations of point *x* of 6A. 6D, a diagram to show all the possible neurons and synaptic connections which might have been concerned in the production of the response deficit at point *x* of 6A.

ing stimulus by 0.6–0.7 msec., the fastest antidromic impulses and the testing impulses in the primary neurons reached the region of the quadriceps motoneuron pool at the same time. This time is indicated by the arrow in Fig.

6A. Inhibition (point x) was already pronounced when the testing impulses arrived very slightly after the conditioning volley. Thus inhibition was first induced when an antidromic volley arrived approximately simultaneously with the testing impulses which fired the tested motoneurons after a single synaptic delay. When the antidromic impulses arrived a little earlier, they produced a greater inhibition; but when they arrived later, no facilitation was apparent.

Conditioning of the discharges of some motoneurons by antidromic volleys in other motoneurons also occurs when the conditioning and tested motor nerves are not branches to the same muscle or muscle group (Table 1). Although it is apparently necessary that the two pools of motoneurons occupy the same segmental levels of the spinal cord, their cell bodies need not lie in the same portion of the ventral horn. The effects for most pairs of nerves are relatively small. Also, as never occurs when the two motoneuron groups occupy the same place in the ventral horn, facilitation is sometimes observed.

In Fig. 7 are shown the conditioning curves obtained in one illustrative experiment in which the antidromic volleys and tested motor discharges occupied the motoneurons of the tibial, peroneal and hamstring nerves. The segmental distribution of the motoneurons of each of these three nerves was determined at the end of the experiment by measuring the size of the motor volleys set up in each nerve by stimulation of the various lumbar and sacral ventral roots. The results are tabulated in Fig. 7D. The comparative similarity in the axial distribution of the motoneurons supplying the three nerves stands in contrast with the cross-sectional segregation of the three groups of cell bodies (Fig. 7E, after Marinesco). In confirmation of Forbes *et al.* (1933), an antidromic volley in the peroneal nerve did not significantly condition reflex discharges into the tibial nerve (Fig. 7A). However, an antidromic volley in the tibial did affect subsequent reflex discharges into the axons of the peroneal nerve. It produced a prolonged period of facilitation of the two-neuron arc discharge to an afferent volley (Fig. 7A, also 8). The facilitation appeared only when the tested impulses followed the conditioning volley by several milliseconds. Initially slight inhibition, which was more apparent in other experiments, occurred. An antidromic volley in the hamstring nerve inhibited the motor discharge into the peroneal, and vice versa (Fig. 7B). An antidromic volley in the tibial likewise inhibited motor discharges into the hamstring nerve, but in the reverse relation the initial inhibition gave way to prolonged facilitation (Fig. 7C).

The conditioning effect of an antidromic volley in one group of motoneurons is not dependent upon the origin of the impulses that serve to excite the tested motoneurons. A shock applied to the ventral columns excites fibers which produce, after a single synaptic delay, a discharge of motoneurons located 2 to 3 cm. caudad of the stimulating electrodes (Lloyd, 1941a). The collaterals which the ventral column fibers send to the ventral horn are oriented, on the average, in the direction opposite to the reflexo-

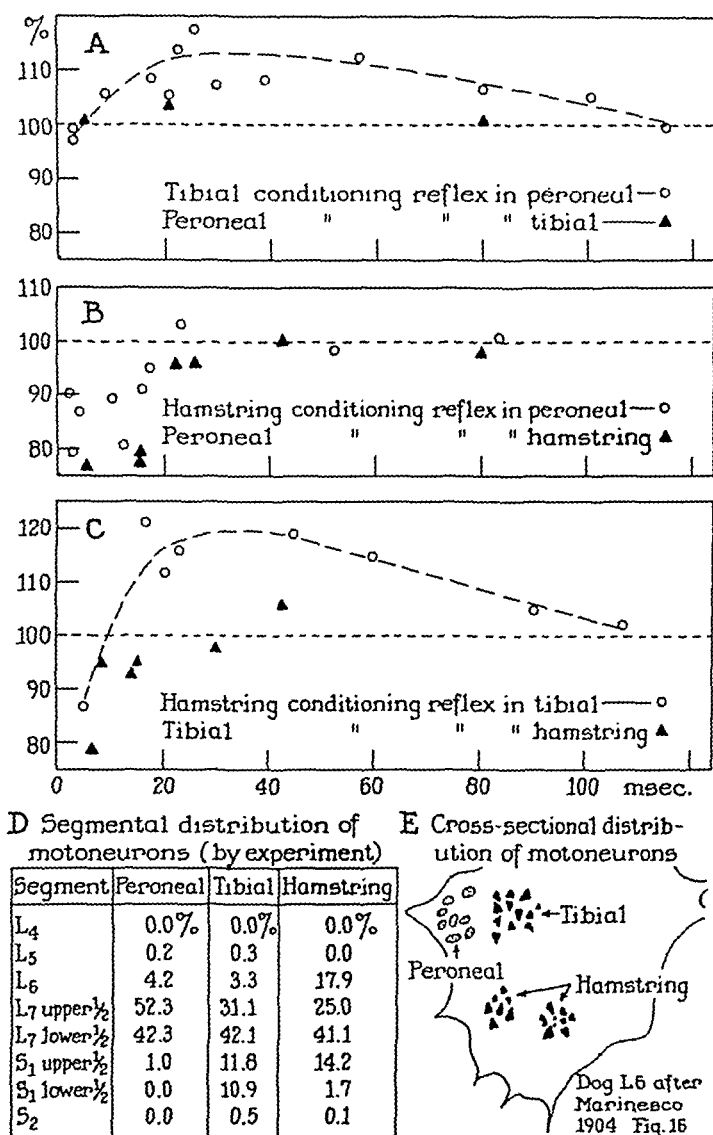


FIG. 7. The interaction between motoneuron discharges and antidromic volleys in the tibial, peroneal, and hamstring nerves of a cat under light anesthesia (Nembutal). The sacral cord had been transected, and all dorsal roots as far cephalad as L3 were severed on the tested side. The motoneuron discharges ("reflexes") were evoked by stimulation of the dorsal columns at L4 with one or two shocks. A, antidromic volley in the tibial nerve conditioning the motor discharge into the peroneal (○); vice versa (▲). B, antidromic volley in the hamstring nerve conditioning the discharge in the peroneal (○); vice versa (▲). C, antidromic volley in the hamstring nerve conditioning the discharge into the tibial (○); vice versa (▲). D, the segmental distribution of the motoneurons of the tibial, peroneal, and hamstring nerves of this preparation. E, the positions of the motoneuron pools of the tibial, peroneal, and hamstring nerves in the cross-section of the ventral horn (after Marinresco). The cell bodies of the peroneal motoneurons show retrograde degeneration.

motor collaterals of the primary afferent neurons. Yet the conditioning effects of an antidromic volley in one group of motoneurons upon the discharges of another group are the same whether the tested motoneurons are excited by ventral column volleys or by impulses in primary afferent neurons. The records of Fig. 8 demonstrate this fact for both inhibitory and facilitatory effects.

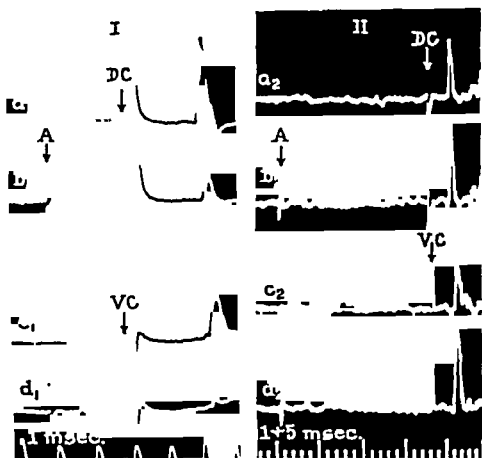


FIG 8 The conditioning by antidromic volleys of reflexes initiated by testing impulses in primary afferent fibers (*a, b*) and in ventral column fibers (*c, d*). Column I, same experiment as Fig 6. Conditioning volleys and tested motor discharges in two nerves to the quadriceps. The tested discharges are inhibited. Column II, conditioning antidromic volleys in the tibial nerve, tested discharges in the peroneal. The tested discharges are facilitated. From an experiment on a cat under light anesthesia (Nembutal). The cord was transected at L1 and in the caudal region, all dorsal roots on the tested side of the isolated lumbar and sacral segments were severed. The conditioning produced by the antidromic volleys is similar, whether the tested discharges are set up by ventral column activity or impulses in primary afferent neurons.

The magnitude of the conditioning effect, although not its qualitative nature, is dependent upon the size of the tested motor discharge. As Tables 2 and 3 show, the larger the tested discharge, the less is the fractional inhibition or facilitation produced by a fixed conditioning volley. This is because the actual number of motoneurons removed from or added to the motor discharge by the conditioning volley increases relatively little. The affected motoneurons may be assumed to be those stimulated approximately at threshold by the testing volley.

Table 2. Motor discharge in nerve to tibialis anticus, set up by stimulation of dorsal column at L5 and conditioned by a preceding antidromic volley in nerve to gastrocnemius. Dorsal roots cut. IV-13-40

Relative size of tested 2-neuron arc discharge into nerve to tibialis anticus	$\frac{\text{Size of conditioned discharge}}{\text{Size of unconditioned discharge}} \times 100$	Per cent facilitation	Relative number of facilitated motoneurons
1	126	26	1.0
6	105	5	1.15

Table 3. Motor discharge in nerve to semitendinosus, set up by stimulation of the dorsal column at L5 and conditioned by an antidromic volley in a branch to the biceps; stimulus interval, 5.5 msec. Dorsal roots cut. IV-16-40

Relative size of the tested 2-neuron arc discharge into nerve to semitendinosus	$\frac{\text{Size of conditioned discharge}}{\text{Size of unconditioned discharge}} \times 100$	Per cent inhibition	Relative number of inhibited motoneurons
1	64	36	1.0
2	80	20	1.1
4	88	12	1.3
20	96	4	2.2

DISCUSSION

It is most unlikely that antidromic conditioning volleys in the present experiments significantly altered the testing volleys in fibers of either the primary afferent neurons or the ventral columns before the impulses reached the ventral horn. For a large safety factor is associated with the propagation of impulses in axons (Hodgkin, 1937; Tasaki, 1939); and, except in the ventral horn, activity of the conditioning motoneurons produces very little current flow which might polarize axons and block conduction. Similarly, there is no evidence that impulses once initiated in the tested motor axons are blocked by antidromic volleys in other axons (page 174). An antidromic volley produces its conditioning effect either by altering the excitability of neurons to synaptic stimulation or by altering the stimuli delivered to post-synaptic elements by the testing impulses in axonal terminations.

It has been shown that activity in one group of motoneurons can condition the activity of another group (Fig. 4 to 8). The initial effect is a response deficit, which is present when the conditioning volley reaches the ventral horn at the beginning of the synaptic delay at the tested motoneurons (point *x* of Fig. 6A). Available for the explanation of these facts are: (i) the arrival of conditioning impulses at the terminal knobs of recurrent collaterals, and (ii) other effects not primarily dependent upon synaptic associations. The present data do not permit a complete resolution of the mechanisms for the conditioning. Some delimitation, however, is possible for the mechanism of the initial inhibitory effect. Inhibition such as that at

point x of Fig. 6A cannot be explained by the sequelae of detonator excitation associated with the arrival of conditioning impulses at the terminals of recurrent collaterals, as shown in hypothetical form at 4 and 5 of Fig. 6D, because detonator effects at these terminals would be expected to sum with those of testing impulses arriving simultaneously at endings 2 and 3 to produce facilitation. It is, therefore, necessary to conclude that the well-known detonator (excitatory) process associated with the arrival of impulses at synapses is not the only mechanism by which an active neuron can affect other nerve cells. This conclusion is corroborated by recent observations on inhibition in which the conditioning volleys occupy primary afferent fibers (Lloyd, 1941b).

The role played by the recurrent collaterals of spinal motoneurons is not known. Hence any explanation for the antidromic conditioning (inhibition, or as the case may be, facilitation) which involves the collaterals is purely speculative. Of the other possible mechanisms there comes to mind most prominently the polarizing action of electrical currents. Currents are in fact set up in the ventral horn by the activity of the conditioning motoneurons (Fig. 6C), and it is known that flow of current through the cord alters the size of testing two-neuron arc discharges (Renshaw, 1940). The only question is whether the currents set up in the cord by the conditioning motoneurons are large enough to produce the effects observed.

For conditioning to occur, the conditioning and the tested motoneurons must lie at the same segmental levels of the cord. A variety of curves is found when the two groups of motoneurons occupy the same axial position but different parts of the cross-section of the ventral horn (cf. Fig. 7). It is obvious that the observed inhibition and facilitation, whatever their basis, must depend upon details of the anatomical substratum in the ventral horn which are as yet unknown. The conditioning effects are not obviously related to reciprocal innervation (cf. Table 1).

The reduction of the tested discharges of motoneurons by their active neighbors in the same pool (Fig. 5) has approximately the same time course as the subnormality of the activated motoneurons to synaptic stimulation (Eccles, 1931; Gasser, 1939; Lorente de Nó, 1939). Thus the firing of some motoneurons in any nucleus produces a decrease in the responsiveness of most or all the motoneurons in the pool. Thereby the susceptibilities of the motoneurons to firing by premotor neurons would be synchronized.

The effects produced by a centripetal volley in a mixed nerve upon reflex discharges into the motor axons of other nerves have commonly been assumed to be referable entirely to impulses in primary afferent axons. The present findings emphasize that effects produced by the antidromic impulses in the motor axons cannot be disregarded.

SUMMARY

An antidromic volley in a group of motoneurons produces a small centrifugal discharge from the spinal cord into some of the motor axons which

carry the antidromic impulses. No centrifugal impulses appear in the axons of other motoneurons. The centrifugal impulses appear to be repetitive discharges set up at some central portion of the motoneurons, rather than reflex discharges synaptically excited through recurrent collaterals.

Antidromic volleys do, however, condition synaptically excited discharges of other motor cells. Inhibition typically occurs if the tested and the conditioning motor nerves are branches to the same muscle or muscle group. The response deficit then reaches its maximum when the conditioning antidromic volley arrives at the cord 2 to 4 msec. before the tested motor discharges are set up. The amount of inhibition then gradually declines. It disappears when the antidromic volley precedes the tested discharge by ca. 50 msec.

A particularly significant feature of the inhibition is its early onset. A response deficit is present if the antidromic volley reaches the ventral horn simultaneously with the testing impulses which fire the tested motoneurons after a single synaptic delay. This finding cannot be explained on the assumption that the only effect which an active neuron exerts upon other neurons is the detonator excitation produced by the arrival of impulses at synapses.

Conditioning also occurs when the antidromic volleys and the tested motor impulses occupy the nerves to different muscles or muscle groups. Both facilitation and inhibition have been observed. Facilitation usually follows a brief initial period of inhibition. Maximal facilitation is attained when the conditioning antidromic volley precedes the tested discharge by ca. 25–30 msec. It then declines and disappears only when the interval between conditioning and tested volleys exceeds 100 msec.

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A DIRECT CENTRAL INHIBITORY ACTION OF DROMICALLY CONDUCTED IMPULSES

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FIBERS, or synaptic endings of fibers, having an inhibitory action, have often been invoked to provide a mechanism by which to explain central inhibition. There has been no direct evidence to support the contention that such fibers or endings exist in the mammalian central nervous system (Gasser, 1937). Furthermore, subnormality (Graham, 1935) of central neurons serves as a mechanism to explain central inhibition without employing other than known processes, provided central neurons are activated prior to the appearance of inhibition (Gasser, 1937). Activation of central neurons is almost inevitable if the central latency of inhibition is greater than the minimal synaptic delay of approximately 0.5 to 0.6 msec. (Lorente de Nó, 1935, 1938). Therefore, to postulate an active inhibitory process requires a demonstration of inhibition under circumstances which preclude the possibility of subnormality in accounting for a response deficit. In effect, the paths taken by the inhibitory action and the necessary testing excitatory action must not have elements in common before impinging upon the final common path (the motoneurons); and motoneurons must not have been discharged by the conditioning impulses, for as Renshaw (1941) has shown by antidromic conditioning, active motoneurons exert an inhibitory action on adjacent motoneurons, which begins without measurable latency and parallels the subnormality of the active motoneurons. The general argument that forces the establishment of these rigid criteria has been clearly stated by Lorente de Nó (1936).

In the experiments presented here the testing excitation was derived from dorsal root collaterals to motoneurons (Cajal, 1890, 1894) which mediate the two-neuron arc discharge (Renshaw, 1940), or from the tract fibers of the ventrolateral columns, which discharge the motoneurons with a single synaptic delay when stimulated within approximately 3 cm. of the motoneuron pool (Lloyd, 1941). The inhibitory effect to be discussed is exerted between the time of arrival of primary afferent impulses (in fibers other than those available to the testing shock), and the time at which the first *de novo* impulses are initiated in central neurons. Hence the rigid conditions imposed above are satisfied in the present experiments.

Cats were used in all experiments. They were lightly narcotized with Dial (approximately 0.5 ml./kilo), after which the lumbosacral spinal cord was exposed and the appropriate roots were carefully prepared for stimulation and recording.

Inhibitory action. Figure 1 illustrates the direct inhibitory action of primary afferent impulses on motoneurons. The impulses exerting the inhibitory action were initiated by a single shock delivered to the sixth lumbar

(L6) dorsal root. The inhibited motoneurons were those that contribute axons to the first sacral (S1) ventral root. A test of the excitability of these motoneurons was obtained by means of single shocks delivered to the S1 dorsal root. The early impulses discharged into the S1 ventral root in response to the S1 dorsal root shock are mediated without the intervention of interneurons. In Fig. 1A and I show the response of the motoneurons to the testing S1 dorsal root shock in isolation; the two-neuron arc discharge is identified by an arrow. In Fig. 1B the conditioning L6 shock and the testing S1 shock were delivered simultaneously with a notable decrease in the two-

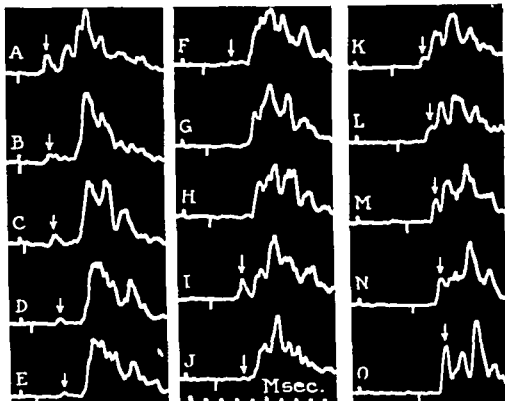


FIG. 1. Inhibition of earliest reflex discharge. Conditioning shock delivered to L6 dorsal root. S1 motoneurons tested by ipsilateral S1 dorsal root shock. The early reflex discharge elevation, recorded from the S1 ventral root, is identified by an arrow except in G and H, where this elevation cannot be clearly distinguished. A and I are motoneuron responses to the test shock in isolation. Further details in text.

neuron arc discharge. When the S1 shock succeeds the L6 shock by increasing intervals, inhibition of the two-neuron arc discharge progresses to completion during the second millisecond (Fig. 1, C to H), after which recovery occurs and inhibition passes over into facilitation (Fig. 1, J to O). In other experiments, inhibition was maximal at stimulus intervals of 1 msec. or less.

Figure 2A shows in graphical form an experiment similar to that illustrated in Fig. 1, but in another preparation. The amplitude of the two-neuron arc discharge is plotted as ordinates against the interval between the two shocks, as abscissae. The combination of roots used was the same as in Fig. 1. At the zero time abscissa the two shocks are simultaneous. At points to the left of zero the testing S1 shock antecedes the conditioning L6 shock. At points to the right of zero this relationship of shocks is reversed.

Inhibition of the two-neuron arc discharge begins when the impulses in the two dorsal roots are approximately synchronized, and it progresses for slightly less than 1 msec., after which the discharge increases in a regular manner to the normal range and into facilitation. It may be stated with reasonable surety that the upswing in the motoneuron discharge, starting just before 1 msec. has elapsed, coincides with the arrival at the motor nucleus of the excitatory impulses of the first internuncial relay. It is clear, therefore, that the inhibition described here begins without measurable latency and achieves considerable intensity before any relayed impulses are initiated in the spinal cord. The total duration of the inhibitory action is not measurable, since it may be curtailed or obscured by the arrival of the relayed excitatory actions. The dorsal root shock threshold for the inhibitory effect has been found to coincide with that of the alpha fibers of the dorsal root.

The records shown in Fig. 2 (B to M) are similar to those used for constructing the curve 2A, and are taken from the same preparation. They were, however, recorded on a slower time axis. Figure 2B shows the motoneuron response to the conditioning L6 dorsal root shock, beginning after some 3 msec. latency. Figure 2C is the motoneuron response to the S1 dorsal root testing shock. The earliest reflex volley, which is used as an indicator, is marked by an arrow. In Fig. 2D, the L6 and S1 shocks are applied simultaneously, while in Fig. 2 (E to M) the testing S1 shock follows the L6 shock by increasing intervals. The inhibition and subsequent facilitation of the two-neuron arc discharge appears as in graph 2A.

Locus of inhibition. It might be supposed, since dorsal root fibers have been used for the initiation of both the conditioning and the testing impulses, that "blocking" of impulses could occur in the dorsal columns (Barron and Matthews, 1935, 1936), presumably at the collateral junctions. On the same supposition, "blocking" could occur at the junction of collaterals to the dorsal horn nuclei, or to the intermediate nuclei. Finally, without prejudice as to its nature, the inhibitory effect could be located within the nexus of the *motoneurons themselves*. The parallel orientation of pathways from neighboring dorsal roots, which permits a multiplicity of possible loci for interaction, may be avoided by substituting a ventrolateral column test volley for the dorsal root test volley.

The collaterals of ventrolateral column fibers have a general orientation in the opposite sense with respect to the central grey substance of the ventral horn. Furthermore, impulses in the two sets of collaterals reach common ground only within the confines of the motor nucleus at the time intervals at present under survey. Therefore, if inhibition of the motoneuron response to a ventrolateral column volley parallels the inhibition of motoneuron response to a dorsal root volley, the only acceptable locus for the inhibitory action under discussion is within the motor nucleus. Figure 3 shows that this parallelism holds.

Figure 3A is the response of the S1 motoneurons to a single S1 dorsal

root shock. In 3B to 3F the S1 dorsal root shock is preceded, at increasing intervals, by a single shock to the L6 dorsal root, the response to which in isolation is seen in 3G. Figure 3H is the response of the same S1 motoneuron pool to a single ventrolateral column shock applied within 3 cm. of the motoneuron pool. In 3I to 3M, the ventrolateral column shock is preceded by the

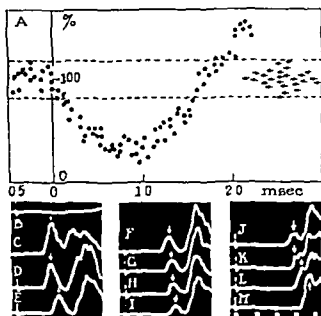


FIG 2 Inhibition in another preparation A, graph showing decrease in amplitude of two-neuron arc discharge, expressed as percentage of control values, and plotted against the time interval between conditioning and testing shocks. The arrows show the amplitude values for 26 control observations, the spread of these values being expressed by the broken lines. B to M are observations similar to those from which graph A was constructed. B, motoneuron response to the conditioning L6 shock. C, response to testing S1 shock. In D to M the S1 shock follows the L6 shock at increasing intervals. The two-neuron arc spike potential is marked by an arrow throughout. Time in 1 msec intervals below record M.

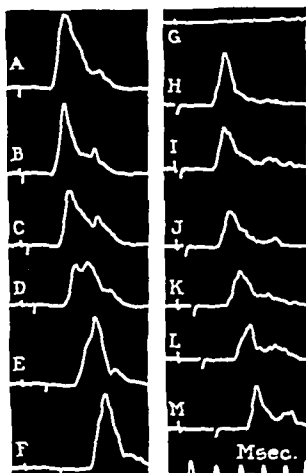


FIG 3 The left-hand column shows inhibition of motoneuron response to a dorsal root shock, while the right-hand column shows inhibition of motoneuron response to a ventrolateral column shock. The responses to the two test shocks in isolation are seen in A and H respectively. G is the response to the conditioning shock in isolation.

L6 dorsal root shock at increasing intervals. All of the observations of Fig. 3 were made on a background of internuncial activity occasioned by an earlier column shock of subliminal strength for motoneuron response. Such an intensified background is not a necessary condition for the performance of the experiment, but it does show that facilitated test responses are subject to the inhibitory action, as are test responses obtained from the "resting" spinal cord.

Since all other circumstances are identical, the observations 3H to 3M with the use of column test volleys may be compared directly with the observations 3A to 3F, with the use of dorsal root test volleys. By this direct

comparison it is seen that the inhibitory action of the L6 impulses is similarly revealed by the dorsal root test shock and by the ventrolateral column test shock. Therefore, the dorsal root impulses must exert their inhibitory action at the motoneuron, or in the immediate neighborhood of the motoneuron.

Table 1. Spatial distribution of conditioning effects in regions at and caudal to entry zone of selected dorsal roots

Source of conditioning activity	Motoneurons tested	Effect of conditioning activity during first msec.
Ipsilateral L5	S1	0 Fig. 5A
Contralateral L6	Lower L7	0 Fig. 4B
Contralateral L7	L7	0
Contralateral L6	S1	0
Ipsilateral L5	L7	Progressive inhibition
Ipsilateral L6	S1	Progressive inhibition as in Fig. 1, 2, and 3
Ipsilateral L6	Lower L7	Facilitation passing into inhibition. Fig. 4A.
Ipsilateral L7	S1	Facilitation passing into inhibition
Upper half Ipsilateral L7	S1	Decreasing facilitation as in Fig. 5B.
Lower half Ipsilateral L7	S1	Strong facilitation as in Fig. 5C.
Upper half Ipsilateral S1	S1	Strong facilitation

Table 1 shows the effect of impulses in dorsal roots which are orientated anatomically in several ways with respect to the tested motor nuclei. Several of the results tabulated are presented in detail in Fig. 4, which compares (A) the conditioning effect of an ipsilateral shock with that (B) of a symmetrical contralateral shock, and in Fig. 5 which shows the conditioning effect of impulses in various ipsilateral rootlets arranged anatomically at several distances cranial to the tested motoneurons. It is clear from Table 1 and Fig. 4 and 5 that inhibitory actions, as well as excitatory actions, of primary afferent fibers are exerted in levels which are supplied directly by the parent descending collaterals of those primary afferent fibers. This has

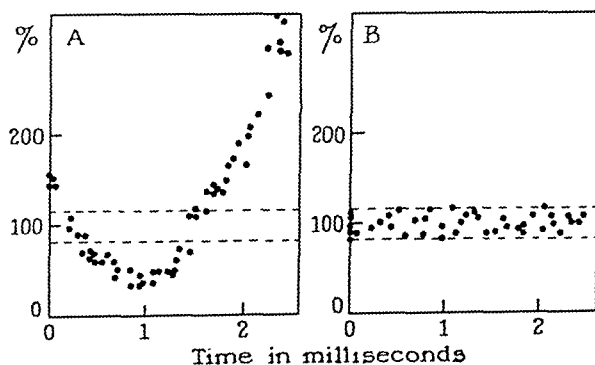


FIG. 4. Graphs constructed as in Fig. 2A. The motoneurons tested are those sending axons to the lower half of the L7 ventral root. In A the response of these motoneurons is conditioned by an ipsilateral L6 dorsal root shock. In B, a contralateral L6 shock is substituted for the ipsilateral L6 conditioning shock.

been confirmed by direct observation of the conducted intramedullary spike potential. It is impossible, because of the lack of exact anatomical knowledge, to state with complete confidence that the motoneurons at all the levels at which effects are demonstrable are in synaptic relation with collaterals from the primary afferent fibers stimulated by the conditioning shock. However, with the reservations enforced by imperfect anatomical knowledge it appears probable that the fibers which cause an effect, whether the effect be excitatory or inhibitory, enter the nucleus in which that effect is exerted.

Figures 1, 2 and 4 show that, although the inhibitory effect is well established before the time at which the first postsynaptic elements can be activated, it lasts beyond the time at which interneurons are certainly active. In fact, inhibition, established before interneurons are active, may become more profound after the time when interneurons must be active. Therefore, it is probable that some interneurons may exert an inhibitory action in their own right, just as do the collaterals of primary afferent fibers.

Comment. In assessing the specificity of the inhibitory action, it is of interest to compare the excitability changes in Fig 5C, in which S1 motoneurons are conditioned by stimulation of the lower half of the ipsilateral L7 dorsal root, and the excitability changes in Fig 2A, in which S1 motoneurons are conditioned by stimulation of the ipsilateral L6 dorsal root. The curves 5C and 2A are from the same experiment with the use of the same testing volley, the result of a single maximal alpha shock to the ipsilateral S1 dorsal root. Let us now consider a point on the two curves before there is a possibility of initiation of postsynaptic impulses, and late enough to avoid any confusion introduced by difference in conduction time of the several volleys. A point about 0.3 to 0.4 msec on the time scales would fulfill these conditions. At this point of time, the S1 motoneuron pool, under the influence of the sequelae of the L7 shock, discharges approximately twice the number of impulses in the test response volley as it does when not so influ-

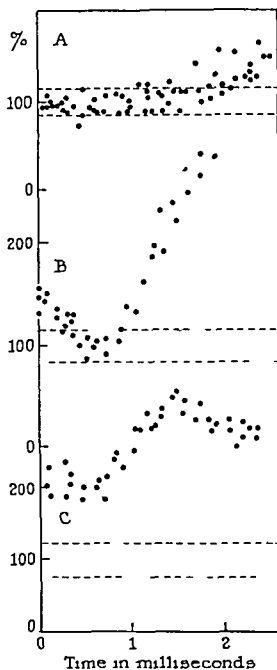


FIG 5 Graphs constructed as in Fig 2A. S1 motoneuron response is conditioned by ipsilateral dorsal root shocks. These shocks were delivered to L5 dorsal root in A, to the upper half of L7 dorsal root in B, and to the lower half of L7 dorsal root in C.

enced. On the other hand, when influenced by the sequelae of the L6 shock, the S1 motoneuron pool discharges but half of the impulses in the test response volley as it does when not so influenced. This difference is a fact that must be accommodated by any hypothesis made to explain direct central inhibitory action. As a first approximation the difference could be accounted for (i) by an important difference in the spatial relationship of impulses with respect to the motoneurons at the time in question, or (ii) by endings of specific effect. It is certain that the inhibition is a direct action without measurable latency. It would seem from this fact that the effect must be electrical. However, with the finer anatomy of the system and synaptic transmission as little understood as they are today, it would serve no useful purpose to pursue speculation further.

SUMMARY

A direct inhibitory action of primary afferent fibers, exerted upon the activation of motoneurons by primary afferent collaterals or ventrolateral column collaterals, has been demonstrated.

The inhibition occurs without measurable latency.

The inhibitory effect is exerted within the confines of the motor nucleus.

It is probable, although not proven, that some internuncial impulses exert a comparable inhibitory action.

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EFFECTS OF LESIONS TO CORPUS STRIATUM UPON SPONTANEOUS ACTIVITY IN THE MALE RAT

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RICHTER and Hawkes (2) reported marked increase in the spontaneous activity of rats subjected to lesions in the frontal portions of the brain. We (1) found that injury to the anterior or posterior cerebral cortex of the male rat sometimes resulted in heightened activity; but in other cases activity decreased after operation. In this study animals sustaining lesions to the frontal cortex suffered varying amounts of invasion to the corpus striatum. However no consistent relationship was discovered between striatal lesions and postoperative changes in activity.

Richter and Hines (3) reported permanent increases in activity resulting from gross removal of the frontal poles of the brain in five macaques. Unilateral removal of the frontal cortex followed by removal of the tip of the striatum regularly produced more marked and precipitous increases in activity than could be elicited by cortical destruction alone. In one animal similar results were obtained with bilateral lesions to the neo-cortex and striatum. These workers concluded that activity is controlled through the prefrontal cortex and the striatum.

Having been unable completely to confirm Richter and Hawkes' results that frontal lesions inevitably produced postoperative increases in the running activity of rats, we have investigated the effects of striatal lesions upon the activity of this animal. It seemed possible that differences between our findings and those of the earlier investigators might be due to variations in lesions inflicted below the neocortical level.

PROCEDURE

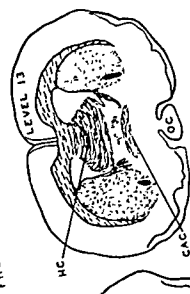
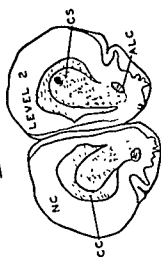
Five male rats 9 months old were placed in the standard activity cages employed in our previous work and in that of Richter and Hawkes. This cage includes a small living chamber adjacent to a 10-inch circular drum. Revolutions of the drum in either direction are recorded on a cyclometer.

After a 30-day preoperative period (which previous results prove ample for high reliability), during which daily readings of activity were taken, each male was subjected to brain operation and returned immediately to the activity cage. Lesions were placed in the corpus striatum by passing direct current into the selected region via a fine wire electrode. Destruction, by electrolysis, is confined to tissue immediately surrounding the uninsulated tip of the electrode.

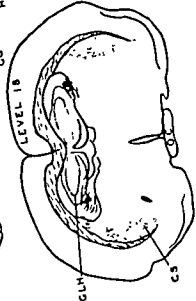
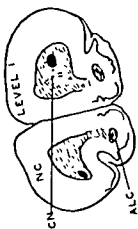
The rats remained in activity cages 50 days after the operation, at which time they were sacrificed, brains were removed, sectioned at 50μ , and stained with thionin.

Results and conclusions. In Table 1 is presented the pre- and postoperative performance of each animal. Rat 1 showed a definite increase in activity following the operation. Numbers 3 and 4 were less active after the operation, and Nos. 2 and 5 exhibited no change in activity.

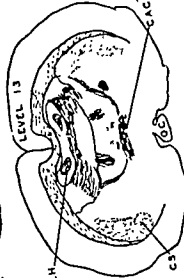
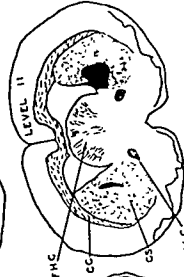
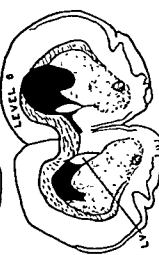
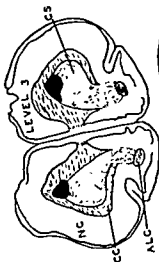
RAT 5



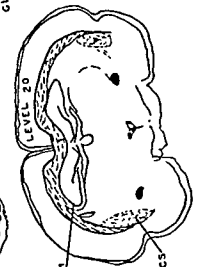
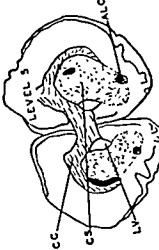
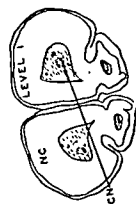
RAT 4



RAT 3



RAT 2



RAT 1

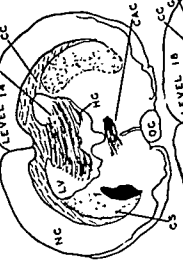
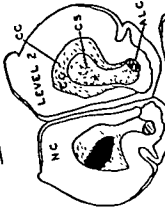


Figure 1 illustrates the lesions inflicted in each case. There is no clear relationship between the amount of destruction or the locus of the lesion and its effect upon activity. Rat No. 1 sustained the largest lesion and showed postoperative increase in activity. Number 3 with the second largest opera-

Table 1 Average revolutions per day before and after partial destruction of the corpus striatum

Days		Rat				
		1	2	3	4	5
Normal	0-4	4	349	324	111	131
	5-9	1	133	285	59	141
	10-14	2	116	241	70	57
	15-19	69	110	145	55	38
	20-24	23	172	106	43	65
	25-29	37	177	82	36	80
Daily average as normal		23	173	198	61	85
Operated	0-4	52	17	64	21	38
	5-9	446	76	96	40	48
	10-14	262	207	58	5	87
	15-19	226	338	63	10	70
	20-24	43	155	37	2	83
	25-29	113	193	74	6	130
	30-34	168	246	117	22	126
	35-39	240	201	103	25	91
	40-44	173	110	91	20	117
	45-49	180	103	83	31	108
Daily average as operate		180	181	78	19	90

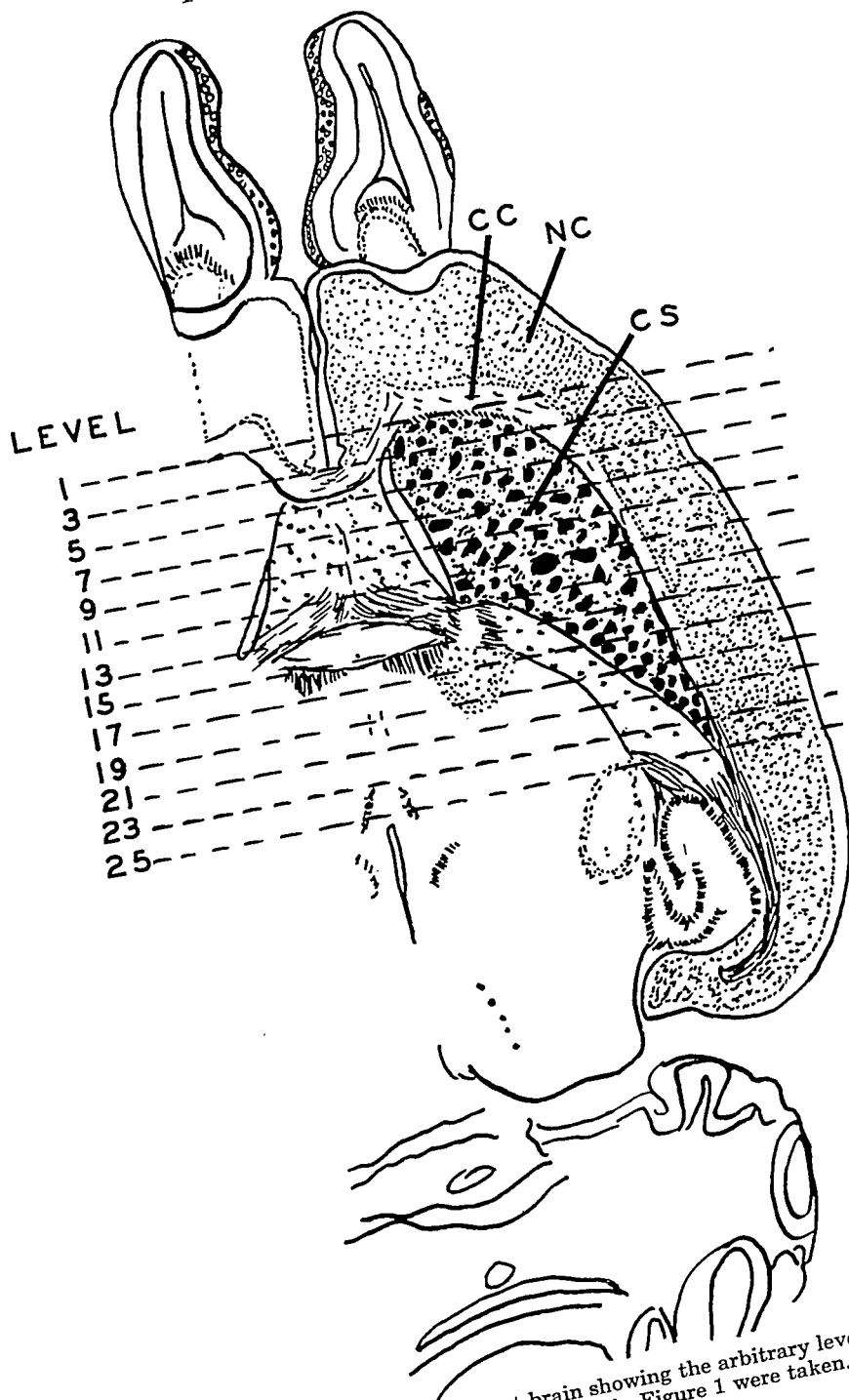
tion was less active as a result of the brain injury. (None of these animals exhibited any marked indications of motor difficulty following the operation. Within three days locomotion appeared to be normal.)

The only conclusion justified by these data is that destruction of the corpus striatum (within the limits herein investigated) has no consistent effect upon running activity in the male rat. There is no clear-cut indication that in this animal the striatum exerts a controlling influence over activity.

FIG 1 Each brain is represented by epidioscope tracings of five cross sections. The first and last sections represent the beginning and end of the lesion, and intermediate sections have been selected at evenly spaced intervals. The "level number" in the upper right corner of each traced section refers to the levels shown in the dorsal view of the corpus striatum sketched on the opposite page

Landmarks in the cross sections are labelled as follows

ALC anterior limb of anterior commissure	GLH granular layer of the hippocampus
CAC crossing of anterior commissure	HC hippocampal commissure
CC corpus callosum	LV lateral ventricle
CN head of caudate nucleus	NC neocortex
CS corpus striatum (lesion in black)	S septum
FHC first fibers of hippocampal commissure	OC optic chiasma



Horizontal section through the rat brain showing the arbitrary levels from which various cross sections traced in Figure 1 were taken.

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FUNCTIONAL ANATOMY OF BRACHIUM PONTIS†

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INTRODUCTION

THE BRACHIUM pontis forms the final neuronal pathway of the cortico-ponto-cerebellar tracts. The anatomic relationships of this system have given rise to numerous and divergent theories concerning its functional significance, but convincing experimental data have been notably lacking. The present study was undertaken in an effort to determine the effects of discrete, anatomically controlled lesions of the brachia pontis. Because of the conflicting results previously reported by both experimental and clinical observers, as well as the possible implications of the anatomic connections of the middle cerebellar peduncle, a broad program of observations was carried out. Psychobiological tests were designed to demonstrate execution and retention of learned behavior patterns, volitional motor skills, and hand-eye coordination. Observations of cerebellar functions, posture, gait, equilibrium, muscle tone, and personality characteristics were included. The periods of study of three animals here reported covered from 5 to 20 months and were concluded by histologic verification of the lesions.

MATERIALS AND METHODS

For studies of learned behavior the animals were trained preoperatively and tested postoperatively on six different types of problem boxes. The first five boxes: rope box, crank box, pull box, hook box, and hook and handle box, were identical with the problem boxes used and described by Jacobsen (1931). The sixth box, sliding door box, had on the side facing the animal a sliding door. By raising the door the animal exposed the interior of the box containing an ordinary tin cup inverted over the food. Peanuts and pieces of orange and banana were used as motivation. Careful quantitative records were kept of the animals' performance.

The coordination, general motor activity, gait and equilibrium were recorded in detailed behavior notes taken daily. Equilibrium was further tested by utilizing a catwalk, a piece of 2" x 4" beam some six feet long. Hand-eye coordination was measured in two ways. A gross method involved suspending a bit of food from a piece of string and swinging it in various planes and at varying speeds within reach of the animal. The other method consisted of placing bits of food on the turntable of a modified Victrola. The accuracy and regularity with which the animals captured the food was then noted for three speeds, slow, medium and fast. Tonus of the extremities, patellar and brachial reflexes, and hopping and placing reactions were measured after the animals had reached an optimum state of relaxation. The different personality traits of the various animals were noted daily in the protocols, and changes in general attitude were carefully watched for after each of the operations.

Operative procedure: Under intraperitoneal nembutal anaesthesia, a large occipitoparietal bone flap was elevated and the dura opened widely. The venous connections to the lateral sinus and the posterior portion of the superior sagittal sinus were fulgurated and

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divided. The occipital lobe was gently elevated, exposing the falx, which was opened by a semilunar incision extending through the edge of the incisura. The anterior aspect of the cerebellum was slowly elevated as fluid was evacuated from the pontine cistern. The petrosal vein was coagulated and divided, exposing the angle formed by the trigeminal nerve with the facial and acoustic nerves at the pons. The middle cerebellar peduncle was identified at this point and a small nerve hook inserted below the inferior margin of the peduncle. The peduncle was completely divided by gentle traction on the hook, without damage to the superior cerebellar artery. The occipitoparietal dural flap was replaced but not sutured, to allow escape of cerebrospinal fluid from the subdural space. The bone flap was replaced and the scalp wound closed.

PROTOCOLS

The three experimental animals herein reported were as follows:

CASE 1 *Mangabey, Male, Wt c 5 kg* The right middle cerebellar peduncle was sectioned March 3, 1937. An attempt to section the left middle cerebellar peduncle on July 7, 1937, resulted in death of the animal.

Microscopic examination revealed that section of the right middle cerebellar peduncle had been virtually complete, with the possible exception of a few of the innermost fibers in the posterior region which might, however, be part of the most anterior portion of the restiform body. No damage to any of the underlying structures in the brainstem could be observed.

CASE 2 *Java macaque Female, Wt c 2.5 kg* This animal had had the right lateral lobe of the cerebellum previously removed by Dr. Fulton. The left middle cerebellar peduncle was sectioned November 18, 1936. From examination of serial sections it appeared that the left middle cerebellar peduncle had not been completely severed, since some of the more medial strands of fibers were seen to be intact. Moreover, on the right side some portions of cerebellar cortex remained, especially near the vermis and in the various sulci.

CASE 3 *Mangabey, Male, Wt c 5 kg* The left middle cerebellar peduncle was sectioned May 25, 1936. The right middle cerebellar peduncle was sectioned September 21, 1936. Abridged protocol notes for this animal will be given as representative of the symptomatology which follows section of the brachium pontis.

First operation on May 25, 1936, the left middle cerebellar peduncle was sectioned.

May 26 Animal is groggy but moderately active. It holds its head markedly to the left. Nystagmus is present, with the quick phase to the right and a slight rotary component. It is unable to use its right leg, and uses both hands in taking food to its mouth. Swallowing appears to be difficult, and the line of the mouth droops to the left.

May 27 Animal is quite active but suffers from obvious vertigo. Falling is to either side indiscriminately. It shows marked dysmetria in use of the right hand, but no tremor is evident. No nystagmus is present. Corneal reflex is absent on the left, and the muscles of mastication are weak on that side. It is rapidly regaining the use of its right leg. Wound is clean, and the flap shows no elevation.

May 29 Animal is tested on problem boxes, all of which it solves in less than 4 sec. It uses either hand swiftly and without errors, save for slight dysmetria. Its legs are used incoordinately in locomotion, the animal mostly pulling itself forward with its forelegs. It holds its head noticeably to the left, and tends to fall to either side. The legs are used fairly well in climbing, in -

June 1 The animal is the hind quarters still sag. Some spiralling and circus movements are still present.

June 8 Animal now uses its legs quite well in walking. There is no tendency to circus movement or spiralling. It can even walk on the hindlegs alone, lifting its feet well with each step, and with good balance.

June 22 The posture of the animal and its use of all four extremities in walking, climbing, etc., are no different now from its preoperative state. The left corneal reflex is still absent. No dysmetria is observable. The general behavior of the animal is similar to its preoperative state: frisky, inquisitive, cooperative.

Second operation on September 21, 1936, the right middle cerebellar peduncle was sectioned.

Sept. 21. 9 p.m. Animal is sitting up in cage. Its spine curves to the right and head is bent far onto the right shoulder. There is some ptosis of the right eyelid. Occasional spontaneous movement of the right arm occurs. Grasp is good with the right hand. When supported in the air, it kicks vigorously with both hind feet.

Sept. 22. Animal holds its head far to the right. There is marked nystagmus, with the quick phase to the left and slightly downward. No tremor is observable in any of the extremities, although there is a slight unsteadiness of the head which simulates a tremor. Wound is clean and shows no elevation.

Sept. 23. Animal's right cornea is cloudy and the right eyelid puffy. Nystagmus is now almost imperceptible. A slight tremor (?) of the head on the neck is still noticeable. There is rather marked dysmetria, but it is hard to evaluate in view of the obvious ocular difficulties.

Sept. 26. Animal is active and inquisitive. It falls heavily when it tries to move about rapidly. Right cornea is now cleared. No nystagmus can now be observed. Some hypometria is present with either right or left hand. Head is held to the right most of the time, but it straightens it to midline when engaged in a voluntary act—e.g. picking up a piece of food.

Oct. 29. The animal walks with a slapping down of the feet to the floor. The feet are not lifted well, and hind quarters sag greatly. Marked circus movement to the right is apparent, and it spirals to the right in climbing. In manipulating objects it tends to work with the left hand while supporting itself with the right arm extended. The spine curves to the right whenever it attempts voluntary movement with either upper extremity. Both legs show some hypotonia on passive manipulation.

Nov. 12. The animal shows absolutely no deficits when tested on the problem boxes, save on the turntable problem in which it is difficult to keep it interested. On the boxes it seems to favor using the right hand more than preoperatively.

Nov. 16. The animal shows the same sort of squat "duck walk" as previously, walking on the full soles of its feet. It now raises its hind quarters about one-half the normal height from the floor. The coördination of hindlimbs with forelimbs is poor. Some spiralling is still present, though circus movements are not particularly apparent. Hopping and placing reactions are normal.

Dec. 9. When the animal holds to the lead chain in walking its legs show a shuffling, incoördinate gait. When the chain is removed and it is allowed to stroll about by itself, it raises its hind quarters well—though still not quite to their normal height—and proceeds more or less like a normal animal. Passive manipulation does not seem to indicate any measureable hypotonia in the lower extremities, yet there is a faltering in the gait and a trembling of the thigh muscles of the right leg when the animal puts weight on it.

Mar. 16, 1937. The animal's walking now differs only subtly from the normal. The hind quarters are not held quite to the normal height, and it tends to place its feet very carefully and to walk on the full sole of the foot. It makes no spontaneous attempts to run, jump, or climb. The coördination of the extremities is now almost, but not quite, normal. There is a subtle lag which is hard to define, and yet its gait is obviously not the easy, rhythmic gait of the normal animal. One gets the impression that the animal must constantly "watch its step," and this may be borne out by the fact that when forced to run, the incoördination between the hindlimbs and the forelimbs becomes more marked, with the result that it loses its balance and falls.

Apr. 29, 1937. Gait remains unchanged since Mar. 16. The animal now seems rather sluggish and lethargic. The problem of finding suitable motivation for the animal in the learned behavior tests grows more and more difficult. The coördination between the hindlimbs and the forelimbs is still delicately out of rhythm, and it uses a somewhat broader base than the normal animal.

Sept. 30, 1937. The gait of the animal seems to show a more exaggerated lack of coördination than at the beginning of the summer. Base seems broader, and it appears to exert more effort in an attempt to keep its extremities correlated in locomotion. It cannot maintain itself on the catwalk, not even when it attempts merely to sit still. There is no constancy to the direction of falling. Tonus seems equal in all extremities. No dysmetria, tremor, or other classical cerebellar signs are observable.

Dec 10, 1937 The animal's condition is similar to that reported on Sept 30, save that all symptoms have increased greatly in severity. It is now very sluggish and expressionless, quite unresistant to handling, deliberate and lethargic in all its behavior. Its facial expression is fixed.

Findings The animal was sacrificed on Dec 15, 1937 and the central nervous system removed for study. On microscopic examination the right brachium pontis appeared to have been completely sectioned without any damage to underlying structures. The lesion of the middle cerebellar peduncle on the left included all the fibers of this system save some dubious strands which mingle with the anterior portion of the restiform body. There was no evidence of damage to brainstem structures on the left side. No other pathologies were noted at autopsy.

Table 1 Symptoms following section of brachium pontis

Symptom	Case 1 Mangabey Rt side only	Case 2 Java (Rt cerebellum removed) Lt br pontis	Case 3 Mangabey	
			Lt	Rt
<i>Transient Symptoms</i>				
Spiralling around long axis of body	++ to rt	+ to lt	++ to lt	++ to rt
Curvature of head and trunk	++ to rt	+ to lt	++ to lt	++ to rt
Incoordination of gait	++	++	++	++
Nystagmus (slow phase)	++ to rt	+ to lt	++ to lt	++ to rt
Dysmetria	+	+	+	+
Tremor	0	0	0	0
Ataxia	0	0	0	0
Hypotonia	+ + left + right	+ + left	+ left + right	+ left + right
Solving problem boxes	Normal	Normal	Normal	Normal
Hopping and placing reactions	Normal	Normal	Normal	Normal
Deep reflexes	Normal	Normal	Normal	Normal
General behavior	Sluggish	Sluggish	Sluggish	Sluggish
<i>Permanent Symptoms</i>				
Hand eye coordination	Normal		Normal	-20 to -30%
<i>Progressive Symptoms</i>				
Equilibratory disorders	0	++	0	++
Incoordination of gait	0	++	0	++
General behavior	0	Sluggish	0	Sluggish

RESULTS

The symptoms occurring after section of the brachium pontis (Table 1) display such constancy of appearance and progression as to indicate the presence of a definite syndrome. The symptoms may readily be divided into three groups: transient, permanent, and progressive.

Transient symptoms

Of the symptoms in this group the following are the most significant:

Spiralling around the long axis of the body appears in conjunction with *curvature of the head and trunk* to the side of the lesion. Such a forced posture

results in circus movements to the operated side, seen prominently during locomotion and other progression movements of the animals. These were the cardinal symptoms of lesions of the brachium pontis as reported by early observers (See Sherrington 1900; Schiff 1858). Until the work of Ferrier and Turner in 1894 there was considerable dispute concerning the direction of these movements. Symptoms of this kind usually disappear by the end of two to three weeks.

Incoördination of gait is quite striking after section of the middle cerebellar peduncle. This incoördination resolves mainly into an inability to correlate the hindlimbs with the forelimbs in locomotion. For a week to ten days after unilateral operations the animals are hardly able to use the hindlimbs at all, locomotion consisting of a crawling with the forelimbs and a dragging and shuffling of the hind quarters. By the end of three to four weeks, however, a unilaterally operated animal can usually not be differentiated from a normal one with respect to gait. In bilaterally operated monkeys the gait never quite returns to normal, but instead suffers a progressive disturbance, as will be discussed shortly.

Nystagmus is usually present for a few days after each operation, the slow phase being toward the operated side. *Dysmetria* may likewise be present transiently. That these two symptoms might be due to local irritation in the neighborhood of the operative site is suggested by the fact that their disappearance often coincides with the recovery of local signs referable to the V and VII cranial nerves.

No tremors in either voluntary or associated movements were noted at any time. Similarly *no ataxia* of the classical cerebellar type was observed. The incoördination of gait previously mentioned did not resemble a true cerebellar ataxia, especially since the upper extremities were spared. Again, the deficit seemed to be mainly a lack of coördination between the hindlimbs and the forelimbs rather than the characteristic cerebellar ataxic syndrome.

The *hypotonia* following section of the middle cerebellar peduncle was the most variable of the postoperative symptoms, although in each case it was significantly greater in the lower extremities than in the upper ones. In Case 1 it seemed most pronounced on the side opposite the lesion, though present bilaterally. In Case 2 the homolateral side was affected almost exclusively. In Case 3 both sides seemed equally affected. The hypotonia tended to disappear concomitantly with the forced postures.

The behavior of the animals in *solving the problem boxes* was in no observable way affected by section of one or both brachia pontis.

Deep reflexes and the *hopping and placing reactions* demonstrated no significant changes after section of the middle cerebellar peduncles.

Following unilateral section of the middle cerebellar peduncle the *general behavior and attitude* of the monkeys were transiently depressed and sluggish. These signs usually disappeared in unilaterally operated animals within

three to four weeks. After bilateral operation the sluggishness tended to progress, and the facial expression became fixed.

Permanent symptoms

A significant permanent symptom was observed in the fact that the bilaterally operated Mangabey (Case 3) showed a *diminution of hand-eye coordination* amounting to some 20-30 per cent below its preoperative norm. No lasting deficit of hand-eye coordination was noted in the unilaterally operated animals.

Progressive symptoms

Of the symptoms in this category three were most striking: an increasing difficulty in coordination of gait, a certain progressive dysequilibrium, and a gradual slowing down and lethargy in general behavior.

After virtual or actual bilateral interruption of the fibers of the brachia pontis (Case 2, Case 3) the locomotion of these monkeys never quite returned to normal. There remained for some 7 to 10 months a subtle faltering and a slight incoordination, of the same general type seen immediately after unilateral section of the peduncle, although greatly less in severity. By the end of approximately 10 months, however, the gait of these bilaterally operated animals was seen to become more and more affected. The gait became progressively more incoordinate (though again not resembling a true cerebellar ataxia) until after the lapse of 12 to 14 months the animals had extreme difficulty in progression even over a flat floor. Sudden sagging of the hind quarters would occur, and the animals tended to fall spontaneously, with no constancy to the direction of falling. Such animals were totally unable to walk along the catwalk, and some *equilibratory disturbance* seemed manifest in the fact that they could not even sit quietly on the catwalk without swaying and falling. Their falling and other equilibratory behavior while walking on smooth flat surfaces appeared more like a vestibular phenomenon than like any form of pulsion.

In those animals in which the brachium pontis fibers were bilaterally interrupted there occurred a *progressive sluggishness*. The facial expression and emotional behavior bore a suggestive resemblance to the human extrapyramidal Parkinsonian syndrome, although at no time were any rigidities, tremors or other abnormal movements noticeable.

DISCUSSION

A tabular outline of experimental cerebellar "syndromes" based upon recent investigations is shown in Table 2. One of the most striking features illustrated in relevant current literature is the remarkable degree of compensation which follows even extensive lesions. However, since neocerebellar function is a relatively advanced phylogenetic acquisition it is not surprising that this should occur. It is probably significant that compensation becomes

less complete as the primate scale is ascended. The relative bilaterality of cerebellar function is likewise notable.

The positive characteristics of cerebellar lesions are tremor, hypotonia, equilibratory disorders, ataxia and disturbances of gait. Tremor has been especially associated with lesions of the dentate nucleus or its efferents in the

Table 2

Lesion	Animal	Syndrome	Investigator
Neocerebellar ablation	Macaca mulatta	Very transient tremor & ataxia	Keller, Roy & Chase, 1937
	Macaca mulatta baboon	Awkwardness, hypotonia & disturbance of gait, 4-6 weeks	Botterell & Fulton, 1938c
	Chimpanzee	"Hypotonia, slowness & slight awkwardness of volitional movement"	Botterell & Fulton, 1936
Cerebellar vermis	Macaca mulatta baboon mangabey	"Serious and enduring disturbances of equilibration."	Botterell & Fulton, 1938b
Nodulus & uvula	Macaca mulatta green monkey mangabey baboon chimpanzee	Lower folia plus nodulus and uvula: disequilibration; nodulus and uvula: disequilibration, rotation of head, rotation in running, jumping, climbing	Dow, 1938b
Inferior cerebellar peduncle	Macaca mulatta	Dysmetria, hypotonia, ipsilateral weakness, diminished reflexes, 2-3 weeks	Ferraro & Barrera, 1935
Superior cerebellar peduncle	Macaca mulatta baboon	Ataxia, tremor, dysmetria & decomposition of movement in ipsilateral extremities	Walker & Botterell, 1937
All three peduncles, unilateral	Macaca mulatta baboon	Ipsilateral ataxia, tremor, disturbances of equilibrium & gait up to 8 weeks	Botterell & Fulton, 1938a

superior cerebellar peduncle. Hypotonia appears after diverse cerebellar injuries, but, in the monkey, is apparently not a significant feature of the chronic picture. Equilibratory disorders are probably related to the vestibular system, especially after lesions to the medial cerebellar nuclei and the flocculonodular lobe. Forced positions and movements are likely in the same category. It is notable that *disturbances of gait* appear with sufficient frequency and duration to constitute a common denominator for neocerebellar lesions. The comparative anatomic development of the neocerebellum with

the assumption of the upright posture is perhaps significant in this connection.

Although the present report is chiefly concerned with behavioral studies, it is perhaps allowable to sketch certain functional anatomical possibilities. The ponto-cerebellar tracts carry numerous fibers whose impulses arise from widespread areas of the cerebral cortex and which finally come to play upon the Purkinje cells of the cerebellar hemispheres. That interruption of these tracts should produce extensive functional loss or diminution of these Purkinje cells seems logical.* It is not unlikely that the functional loss thus produced is more widespread than that following cerebellar cortical lesions previously reported. Cerebellar efferent impulses presumably pass from the Purkinje cells through the dentate nucleus and traverse the dentato-rubro-thalamic tract in the superior cerebellar peduncle. Their final effect may be mediated through the rubrospinal tract or directly upon the cerebral cortex through the thalamo-cortical fibers.

Concerning the question of cerebello-cortical impulses, the observations of Walker (1938) are of interest. Walker has shown that faradic stimulation of the lateral lobes of the cerebellum is reflected in changes of the resting action potentials of the cerebral motor cortex. From his observations Walker formulates the theory that the normal function of the neocerebellum may quite possibly be that of adding to the motor area a certain "dynamic tone." Other oscillographic studies by Dow (1938a) indicate that postural tone in the extensor muscles (e.g. plantaris) of the cat is inversely proportional to the functional activity of the anterior lobe of the cerebellum.

The possibility that the postural afferent systems may be modified by cortico-cerebellar impulses has been suggested. It is uncertain whether the forced postures and movements observed during the first weeks in the experimental animals are indicative of vestibular release or merely related to the operative procedures adjacent to the eighth nerve and vestibular nuclei. It is significant that these postural manifestations were much more enduring than obvious neighborhood symptoms referable to the V and VII nerves. There was no microscopic evidence of injury to the vestibular system.

The relation of the present experimental results to previous theories concerning the functional anatomy of the cortico-ponto-cerebellar system requires brief consideration. No interference with the proper execution of motor formulae (Tilney and Riley 1921) was observable after lesions of this system. Similarly no deficits in the capacities for precise manual function of the forelimbs, nor any lack in learned versus phylogenetically conditioned behavior (Tilney 1928; Yochelson 1936) could be noted. Indeed, if interference with the execution of learned motor formulae were to be brought about by severing the cortico-ponto-cerebellar pathways, it should certainly show up in animals trained in the problem box techniques, for the work of

* Data indicating progressive widespread atrophy of Purkinje cells after lesions to the brachia pontis will be reported in a later paper.

Jacobsen (1931) indicates that these patterns are a function of the areas of the cerebral cortex from which a preponderant number of the corticopontine fibres arise. At no time did any of the animals display states even remotely resembling decerebrate rigidity (Wilson 1920; Warner and Olmstead 1923; Smith 1924; Abbie 1934), nor was there at any time a post-operative condition which appeared as an "akinetic-hypertonic" syndrome (Jakob 1923). Save for slight transient post-operative dysmetria, there were no immediate signs suggesting symptomatology of a more classical cerebellar nature.

It is worth mentioning again that close observation of the terminal incoördination of gait seen in the bilaterally operated animals indicated that the fault lay in *inadequate coördination of the lower with the upper extremities in locomotion*, and that this incoördination bore only a superficial resemblance to a true "cerebellar ataxic gait." The following statement of Walshe (1927) concerning cerebellar phenomena is pertinent: "On the other hand, a disturbance of the cortical process of motor synthesis. . . may well underlie cerebellar ataxia . . . The disorder of some higher grade of coördination involving the participation of the cerebral motor cortex and concerned with the harmonious relationship between component coördinated units of large voluntary movement complexes may conceivably be the basis of the cerebellar symptom complex." This concept of the cortical motor areas as the site of genesis of cerebellar phenomena has been verified by the experiments of Fulton, Liddell and Rioch (1932) and by those of Aring and Fulton (1936).

SUMMARY AND CONCLUSIONS

The effects of section of the brachium pontis were studied in three monkeys (2 mangabeys, 1 Java monkey) over periods of 5 to 20 months. The observations included psychobiological tests (problem boxes) to which the animals were previously trained, determinations of cerebellar functions, posture, gait, equilibrium, muscle tone, and personality characteristics. The operative lesions and their resultant degenerations were verified histologically. The results may be summarised as follows:

1. Neither the retention nor the execution of learned behavior problems was significantly affected by unilateral or bilateral section of the brachium pontis.

2. Unilateral section of the brachium pontis was followed by curvature of the head and spine, spiralling and circus movements toward the side of the lesion; awkwardness of the lower extremities in locomotion, with incoördination between the hindlimbs and the forelimbs; slight hypotonia of both lower extremities. These symptoms disappeared within three to four weeks. A decrement in activity sometimes endured for a somewhat longer time. Transient dysmetria and nystagmus were present for a few days after operation. No tremors in either voluntary or associated movements were noted at any time.

3. Bilateral section of brachium pontis resulted in the same symptoms as the unilateral operation, but enduring awkwardness of gait and diminished general activity were present. The hand-eye coordination was reduced 20 to 30 per cent below preoperative normal.

4. The bilateral operation was followed by a progressive symptom complex consisting of incoordination between the lower and the upper extremities in locomotion, dysequilibrium, and sluggishness of general behavior.

The following implications may be drawn from the results of this study:

1. The cortico-ponto-cerebellar system is not necessary for the performance of precise manual functions or learned behavior.

2. The complete compensation which occurs following unilateral lesions indicates that the cortico-ponto-cerebellar system is capable of bilateral function.

3. Functionally the cortico-ponto-cerebellar system seems to be necessary for the coordinated activity of large movement complexes, such as the relationship between the lower and the upper extremities in locomotion.

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MULTIPLE MOTOR INNERVATION OF THE FROG'S SARTORIUS MUSCLE

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(Received for publication November 25, 1940)

THE FROG's sartorius muscle owes its place as a classical preparation for the study of muscular activity and myoneural transmission to its well-suited structure, being a thin muscle of long parallel fibres and containing at its pelvic end a region of several millimetres which is free from motor nerve endings. In this paper another peculiar property of the frog's sartorius is described, *viz.* that practically all its fibres are provided with at least two motor nerve endings situated in discrete regions, and often concentrated within definite narrow zones, of the muscle.

METHOD

The experiments were made, during January–May 1940, at 20–30°C, on isolated sciatic sartorius preparations of Australian frogs (*Hyla aurea*). The animals were kept in the laboratory in a cold store, at about 14°C. Their sartorius muscles are about 35 mm long, delicate and thin, and resembling in size the sartorius of European *Rana temporaria*, they remained in good condition for many hours during the experiment, even at high temperature. As a rule, the preparation was soaked for an hour in oxygenated Ringer's solution before use.

The preparation was mounted in a moist paraffin wax chamber on platinum electrodes the position of which could be adjusted from the outside. The Pt-wires were attached to ebonite rods sliding, in parallel with the longitudinal axis of the muscle, in ebonite tunnels which were fixed in the paraffin block at either side of the chamber.

The muscle was stimulated, directly or through its nerve, by break induction shocks delivered from a Lucas pendulum. The action potential of the muscle was recorded with a 3-stage condenser coupled amplifier and cathode ray oscillograph. With a rectangular voltage input, the preparation being replaced by moist filter paper, the oscillograph deflection reached 90 per cent of its maximum in 0.1 msec, and declined to $\frac{1}{2}$ in 0.2 sec.

When recording from the sartorius muscle, "lead artefacts" such as described by Bishop, Erlanger and Gasser (1926) and by Bishop and Gilson (1929) are sometimes encountered. (i) When the leads are applied at either side of the nerve entry, fluid and tissue rests attached to this region act as a "false lead" which complicates the record by introducing a small diphasic wave. (ii) A positive initial wave preceding the period of negativity was sometimes recorded when leading from the extreme end of the muscle, or when using submaximal stimulation (cf. Bishop and Gilson, 1929).

In some cases the twitch tension was recorded with an optical isometric myograph, the deflection being read on a scale.

RESULTS

1. SHAPE OF THE ACTION POTENTIAL OF THE FROG'S SARTORIUS

The distribution of motor nerves in the frog's sartorius has frequently been described (*e.g.* Pézard and May, 1937) and is shown diagrammatically in Fig. 1. If recording leads are placed on the pelvic part of the muscle (Fig. 1) and the nerve is stimulated by a maximum induction shock, one would expect to observe a prolonged and complicated action potential made up by

* Carnegie Research Fellow.

individual waves which originate in the various junctional regions and arrive in succession at the pelvic ends of the muscle fibres. Considering (i) that the sartorius consists largely of long fibres running from end to end (cf. section 5), (ii) that the motor endings are scattered over a distance of about 2 cm. and (iii) that the muscle impulses travel at a speed of about 2 m/sec., at 25°C, one might expect to obtain a very complex potential wave with individual spikes separated by as much as 10 msec. It was rather surprising, therefore, to find in a normal muscle, as the result of a maximal nerve stimu-

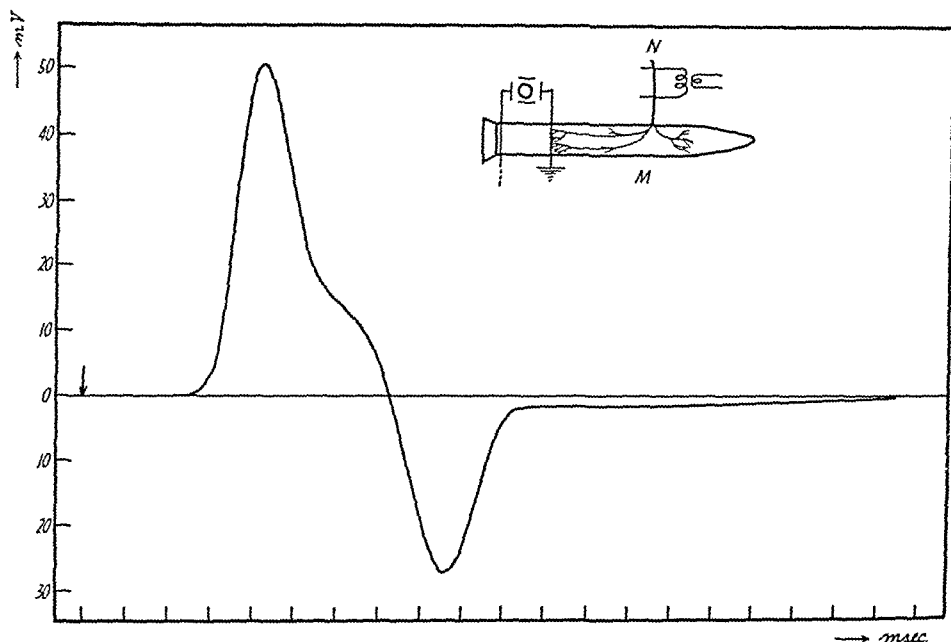


FIG. 1. Diphasic action potential of frog's sartorius at 22°C. Leads at 8 and 0 mm from pelvic end. Nerve stimulus marked by arrow. Inset: Diagram of sartorius (M) with nerve (N) and stimulating and recording leads. Approximate position of main innervation zones is indicated.

lus, a single spike (Fig. 1, 2) as large as, and not longer than, that obtained by maximum direct stimulation (*i.e.* about 50–60 mV. and 4–5 msec. respectively). Obviously the expected late waves starting on the other side of the nerve entry had failed to arrive at the recording electrode, while apparently, in a majority of the muscle fibres, impulses had been initiated nearby. A similar result was obtained when recording from the tibial part of the muscle.

The action potential wave is not always smooth and synchronous as in the cases of Fig. 1 and 2a. Often it has two or three distinct humps (Fig. 2b) indicating that the points of origin of the impulses in different fibres are scattered over a few mm. While the shape of the action potential thus varied from muscle to muscle, and from point to point in each preparation (see

section 3, p. 217 below), the absence of delayed spikes which might have come from the distant parts of the muscle was consistent.

A possible explanation of this phenomenon might be the presence in

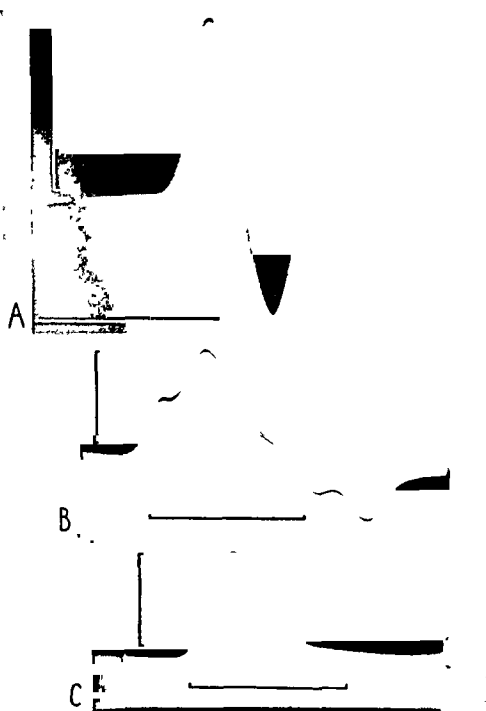


FIG. 2. Action potentials of sartorius muscles. Indirect stimulation. *a*. Diphasic. 20°C. Leads at 7.5 and 4 mm (from pelvic end). *b*. Diphasic. 18°C. Leads at 10 and 0 mm. *c*. Monophasic (by application of isotonic KCl to pelvic end). 18°C. Active lead at 12 mm. Time scale: 5 msec. Volt scale: 50 mV.

most fibres of multiple motor nerve endings, concentrated in discrete "neural" zones. In this way impulses originating at nerve endings far away from the amplifier leads would collide during conduction with those coming from the more proximal junctions, and so could not be recorded. This suggestion seemed to contrast with the current view (Kulchitsky 1924, Fulton

1926) *viz.* that multiple innervation of individual muscle fibres does rarely, if ever, occur. In several ways, however, clear-cut evidence for the correctness of this explanation was obtained.

2. EVIDENCE FOR MULTIPLE INNERVATION

a. Removal of one nerve branch

The nerve supplying the sartorius divides into two main branches, a pelvic and a tibial branch, immediately before entering the muscle. If, for example, the pelvic branch is cut, the spike previously recorded from the

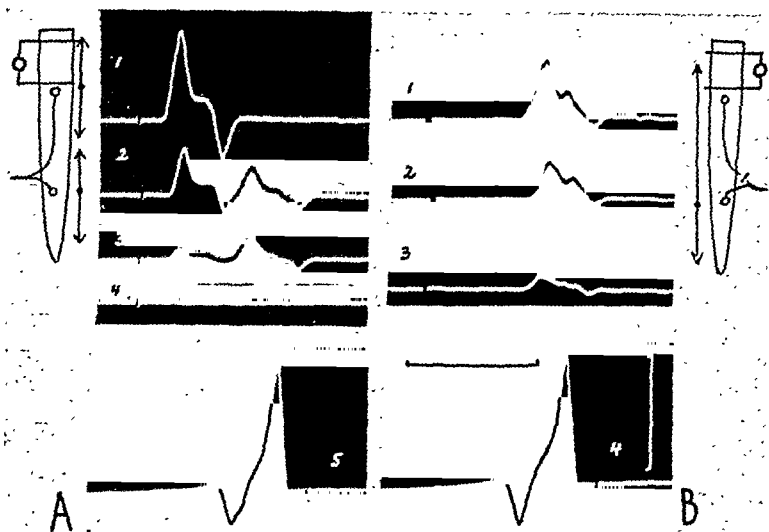


FIG. 3. Effects of partial denervation and of submaximal stimulation. Sartorius, 38 mm long, at 27.5°C. A. Intact nerve supply. A, 1-4: Leads at pelvic region (12 and 2 mm). Strength of nerve stimulus, successively from above, in relative units: 123 (maximal), 103, 100, 99. A, 5: Leads at tibial region (28 and 35 mm; record runs from right to left). Maximal nerve stimulus. B. After pelvic branch has been cut (*cf.* diagram). B, 1-3: Leads at pelvic region (10 and 1 mm). Strength of stimulus: 117 (maximal), 103, 100. B, 4: Leads at tibial region. Maximal stimulus. Time scale: 10 msec. Volt scale: 30 mV.

pelvic end (Fig. 3 and 4) disappears and, in its stead, a delayed spike is observed reaching the pelvic electrodes after a latency of 7 msec., instead of 2 msec. (Fig. 3, B). Obviously, those impulses which start in the tibial part of the muscle, supplied by the intact nerve branch, and which previously had been stopped on their way by collision with the pelvic impulses are now able to travel unhindered to the pelvic end of the muscle. Action potentials recorded from the tibial "intact" portion remain unaltered.

The delayed spikes reached only 20-35 mV in amplitude, *i.e.* about 50-70 per cent of the early spike, an effect attributable to the increase in temporal dispersion. The difference between the spike latencies before and after nerve section varied between 5 and 7 msec. With a conduction velocity of

2-2.5 m/sec., therefore, it appears that many fibres are innervated in 2 discrete "zones" of the muscle, about 12-15 mm. apart.

It is clear that delayed spikes must appear whenever the collision of muscle impulses is abolished or rendered incomplete. This can be achieved in various ways, *e.g.* by cutting one nerve branch as above, by *submaximal*

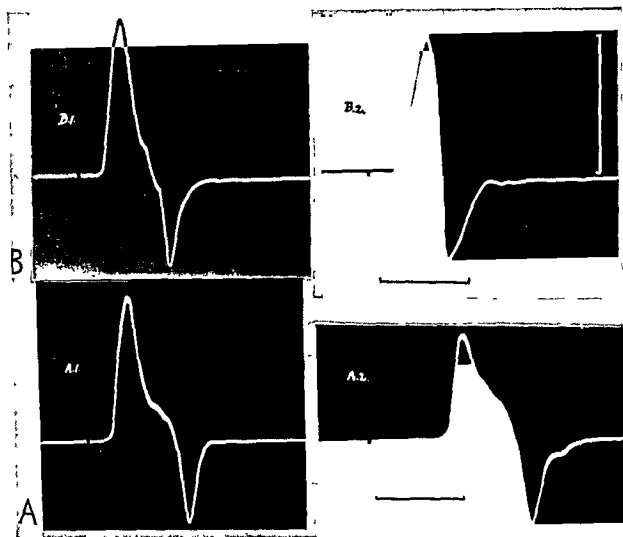


FIG. 4. Effect of partial denervation. 16.5°C. A. Half-denervated sartorius muscle. Pelvic branch had been cut 16 days before the experiment. Maximal nerve stimulus: A, 1: recording from tibial; A, 2: from pelvic region. B. Other sartorius of same frog. Intact nerve supply. B, 1: tibial; B, 2: pelvic recording. Time scale: 10 msec. Volt scale: 50 mV.

nerve stimulation, by partial curarization, and finally by interposing a direct stimulus at a suitable time interval.

b. Submaximal stimulation of the motor nerve

Figure 3 shows action potentials of the sartorius muscle (i) to maximal, (ii) to submaximal stimulation of the nerve. It so happens that in the latter case, the impulses set up at the pelvic and tibial junctions respectively are travelling largely in separate fibres and, therefore, a discrete late spike is recorded, which is not present when all nerve fibres are excited by stronger stimuli. The phenomenon is not always marked; it depends largely upon a chance arrangement, *viz.* upon the presence and frequency of individual

muscle fibres which happen to be supplied, at their respective pelvic and tibial junctions, by *separate* axons of *different* excitability.

If one branch of the nerve is cut, as above, then with all strengths of stimuli only the early spike is recorded on the intact side, and only the late spike on the denervated side.

c. Partial curarization

A condition similar to submaximal nerve stimulation is obtained by weak curarization of the muscle. For this, the preparation was soaked in Ringer's

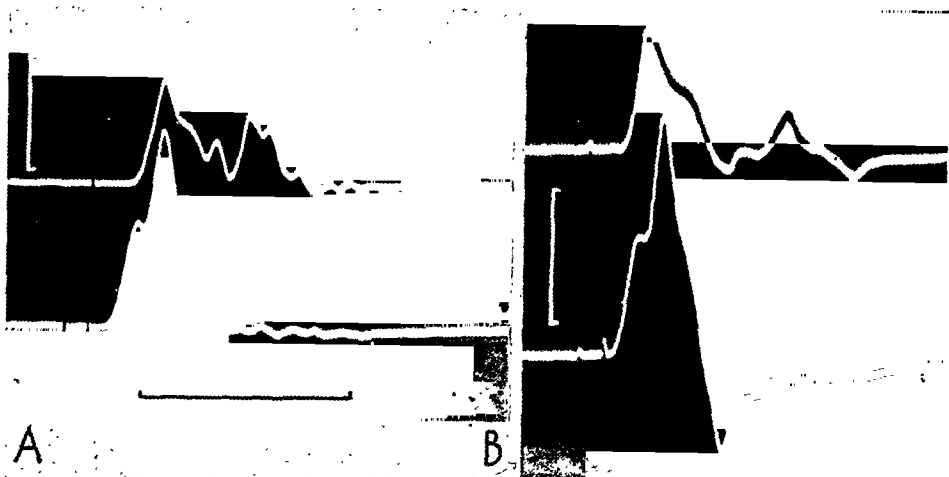


FIG. 5. Partial curarization. Upper records: Single, lower records: double maximal nerve volleys. A. Surface application of curarine (3μ mol/l.) to pelvic part. 27.5°C . B. Muscle soaked in 1μ mol./l. curarine. 26°C . Time scale: 10 msec. Volt scale: 20 mV.

solution containing 1μ mol. curarine per 1 litre. In this way a stage of incomplete neuro-muscular block is attained in which, in many muscles fibres, one junction happens to be blocked, while the other is still functioning, so that many impulses can run along to the other side of the muscle without colliding. Figure 5 shows an example of this kind. The normal action potential is reduced in size and split into 2 discrete spikes, about 7 msec. apart, by weak curarization. If the "distal" nerve branch is cut, the late spike disappears.

There is another way of abolishing the late spike in a partially curarized preparation, namely by sending a second nerve volley down to the muscle at a brief interval after the first. Figure 5 shows that a second stimulus to the nerve applied 1.3 msec. after the first, produces a considerable addition to the early spike, and practically abolishes the delayed spike. On account of neuro-muscular facilitation (cf. Bremer, 1927) the second volley excites at nearly all those junctions which had failed to transmit the first impulse and so the late spike is eliminated by collision as in normal muscle.

Incidentally the second volley has been discharged so early in the refractory period of the muscle that no second impulse could be set up in those fibres which were activated by the preceding volley.

d. "Antidromic" stimulation

The presence of multiple innervation can be demonstrated in another way. The muscle is excited directly by a maximum shock to its pelvic end (*M* stimulus), and a little later, when the muscle volley has travelled beyond the pelvic junctions, a nerve volley (*N*) is sent down. The following situa-

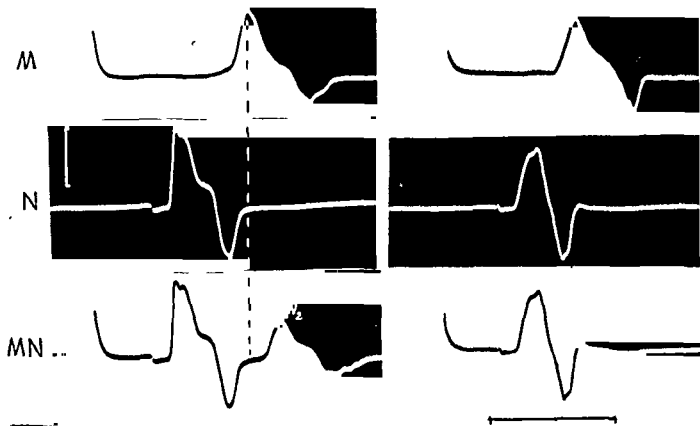


FIG. 6. "Antidromic" stimulation. Muscle 34 mm long. 26.5°C. Leading at tibial region (24 and 32 mm). Direct stimulation at pelvic end (cathode at 3, anode at 1 mm). *M*, direct stimulus. *N*, nerve stimulus. *MN*, combined stimuli, *N* 4.9 msec. after *M*. Left: intact nerve supply. Right: pelvic branch cut. The *M* spike collides with, and is blocked by, the *N* spike (Right, *MN*), but makes way for a late spike (*N*₂), coming from the pelvic region (Left, *MN*). Note the different timing of *M* and *N*₂, and the disappearance of *N*₂ after the pelvic nerve branch has been cut. Time scale: 10 msec. Volt scale: 20 mV.

tion occurs: the muscle volley set up by direct stimulation (referred to below as the "antidromic" volley since it travels from the nerve-free end towards the junctional region) collides, somewhere near the nerve entry, with the impulses coming from the tibial junctions. In the wake of the antidromic volley, the muscle is still refractory, but farther back at the pelvic junctions it has recovered and so another muscle volley, initiated by the nerve, follows behind the antidromic volley and travels unopposed down to the tibial end, where it is recorded (Fig. 6). The antidromic volley has cleared the way for the pelvic impulses.

The size and, within certain limits, also the timing of the late "pelvic"

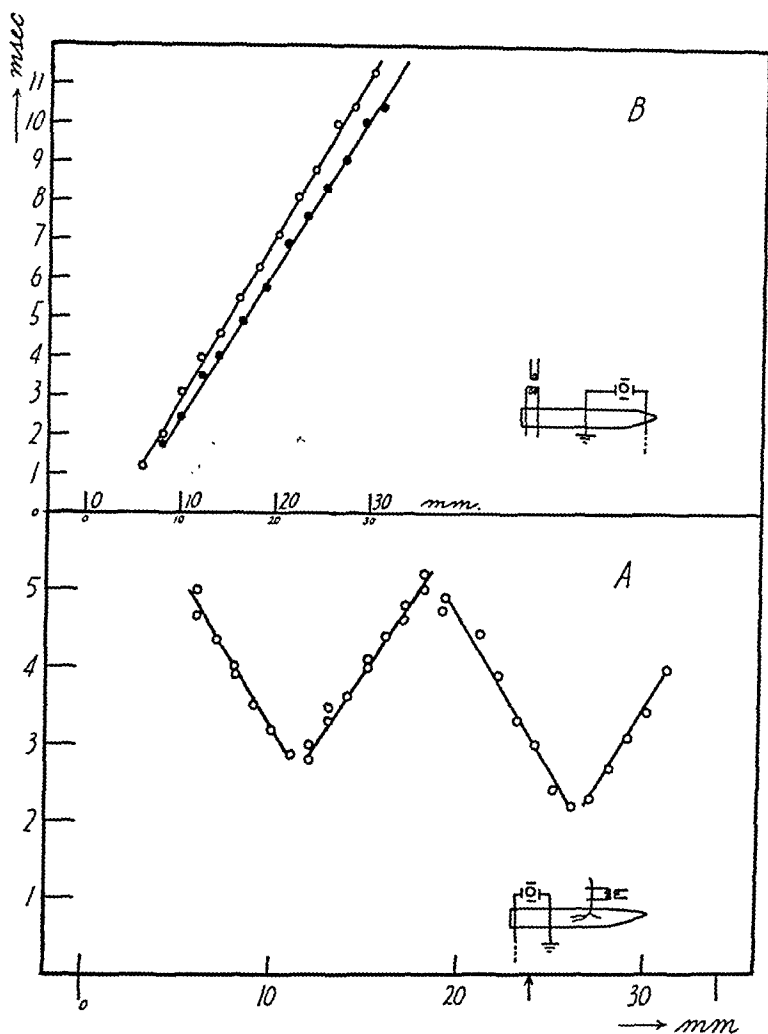


FIG. 7. Relation between shock-peak interval and position of recording lead. 27.5°C. A. Nerve stimulation. Position of nerve entry marked by arrow. Length of muscle 34 mm. B. Direct stimulation. B, 1. Normal. B, 2. After 10 μ mol. curarine. With B, 2, the muscle was stretched to a 6 per cent greater length which probably accounts for the apparent slight increase (10 per cent) in conduction velocity. Abscissae: position of earth lead on muscle, in mm from pelvic end. Ordinates: shock-peak time in msec.

spike depends upon the interval between the *M* and *N* stimuli. If the nerve volley is fired too soon, or too late, *i.e.* before the pelvic, or after the tibial, junctions have recovered from the antidromic volley, the late spike fails to appear. With intermediate intervals, the size of the late spike becomes larger and its latency smaller, as the antidromic volley and the recovery from it, proceeds towards the tibial junctions.

If the pelvic nerve branch is cut, the late spike is abolished (Fig. 6, Right, MN).

3. LOCALIZATION OF MULTIPLE MOTOR ENDINGS

The preceding experiments show the presence of an extensive multiple innervation in the sartorius muscle. Further information, however, is required regarding (i) the number (two or several) of motor nerve endings in individual fibres and their exact position, (ii) the fraction of the muscle fibres concerned.

If the position of the earthed electrode (Fig. 7) is varied along the muscle, systematic changes in shape, size and timing of the spike are observed (Fig. 7-10).

In the following, the position of the earth lead on the muscle is given in millimetres distance from the pelvic end. The grid lead is applied to "0 mm," unless stated otherwise.

From about 8 mm to the pelvic end, the impulses are conducted at a uniform rate of about 2 m/sec. This applies to both indirect and direct stimulation, and to normal as well as curarized muscle (cf. Göpfert and Schaefer, 1938). It is merely a confirmation of the well known absence of motor nerves at the pelvic end.

Beyond that region, one must distinguish between the *beginning* and *summit* of the action potential wave. The start of the action potential ("shock-start" delay) varies only slightly with the position of the recording electrode. Since some motor endings are scattered usually over the whole of the muscle except the pelvic and extreme tibial end (Pézar and May, 1937), the shock-start delay, beyond 6 or 7 mm., depends only upon conduction in the intramuscular nerves which is about 10 times faster than conduction in the muscles fibres (cf. Göpfert and Schaefer, 1938). Thus the shock-start delay is a minimum at the point of nerve entry, increases slightly, at a rate of about 0.05 msec./mm towards the end

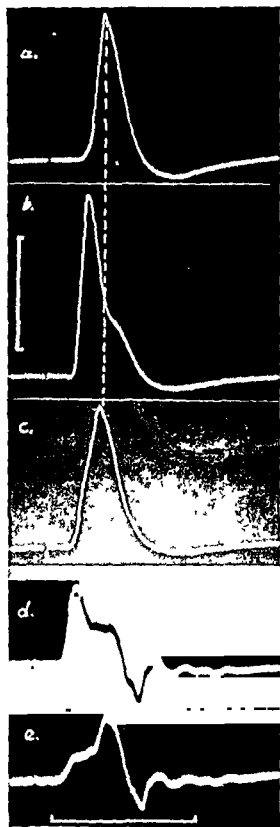


FIG. 8. Changes in form and timing of spike recorded at different positions. *a-c*: 22°C. "Nearly monophasic" (KCl at pelvic end). Positions of active lead, successively from above: 7, 10, 14 mm from pelvic end. Minimum latency at 10 mm. *d-e*: 27.5°C. Diphasic. Position of earth lead at 12 and 19 mm respectively. Time scale: 10 msec. Volt scale: 30mV.

of the muscle until the nerve-free part is reached; from there onwards, it increases at a 10-fold rate.

However, the delay of the summit of the action potential ("shock-peak" delay) varies in a very different fashion (Fig. 7, 8, 10). Figure 7, for example, shows two sharp minima at 11–12 and 26–27 mm, and a maximum shock-peak delay at 18–19 mm, not far from the nerve entry. Obviously, in this

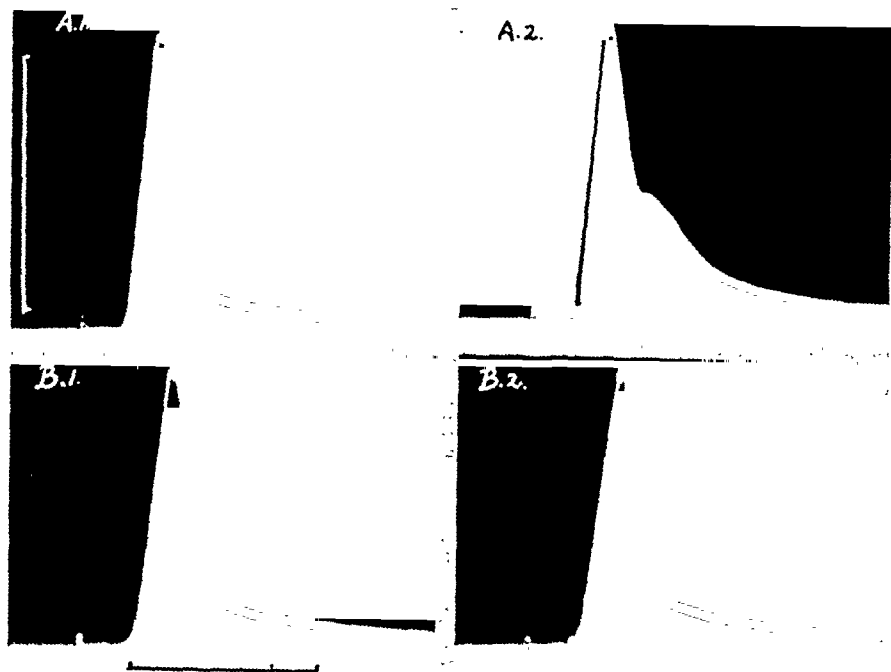


FIG. 9. Effect of early second nerve volley. Monophasic spikes. 18°C. A. Position of earth lead at 10 mm. A. 2: Second nerve volley, after 2.5 msec., adds non-conducted "end-plate potential." B. Position at 12.5 mm, i.e. 2.5 mm nearer the nerve entry. No noticeable addition by second nerve volley (measurement shows an addition which is about 1/30 of that at 10 mm). The spike peak at 12.5 mm is 1.6 msec. later than at 10 mm, corresponding with a conduction velocity of 1.6 m/sec. Time scale: 10 msec. Volt scale: 50 mV.

particular muscle there were two main "innervation zones", at 11 and 26 mm, where volleys of impulses were set up in many muscle fibres proceeding to both sides at a speed of 2.5 m/sec. It is possible to follow them on their way because the position of the spike summit depends, not so much upon the presence of scattered junctions, but upon the arrival of a synchronous volley. It is clear, therefore, that while some motor endings are distributed over the whole length between 11 mm and 27 mm, large fractions are concentrated at definite "neural" zones. The picture is not always as simple as in Fig. 7, usually there are several centres of innervation (see e.g. Fig. 10).

These observations have been confirmed by recording the non-propagated potential changes set up in the junctional region of the muscle fibres by a blocked motor nerve

volley (see Eccles, Katz and Kuffler, 1940) These "end plate potentials" are found only in restricted "neural" zones of muscle, and their positions agree with those of the minimum shock-peak intervals (cf Fig 9)

Taken together with the previous evidence (Section 2) it can be concluded that the two "neural" zones shown in Fig. 7, A separated by a distance of

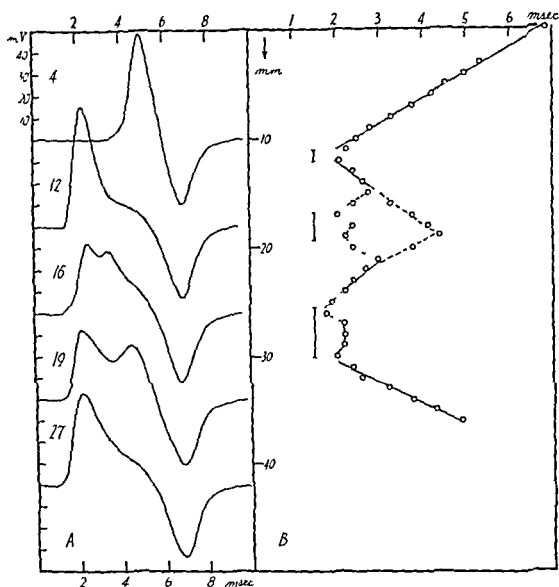


FIG 10 Changes in timing of spike at different positions 26°C A Diphasic spikes Earth lead (successively from above) at 4, 12, 16, 19, 27 mm from pelvic end Grid lead at pelvic end Volt scale 10 mV marks Abscissae interval after nerve stimulus in msec B Ordinates Position of earth lead on muscle, in mm from pelvic end Abscissae Shock-peak interval in msec

15 mm, belong to the same muscle fibres Figure 7 shows also that many fibres are innervated at two regions *only*. Otherwise it would not have been possible to follow the conduction of the spike summit from the 2 "neural" zones to the point of collision at 18 mm. On the other hand, there is no doubt that a good many motor endings are distributed over the intermediate distance between 11 and 27 mm. They might belong to separate muscle fibres, not innervated at the 2 main zones, or they might be additional motor endings on the same fibres.

4. NUMBER OF MULTIPLE MOTOR ENDINGS

There is good evidence that these intermediate motor endings constitute an additional (third, or even fourth) nerve supply for a considerable part of the muscle fibres. This is made probable by the high degree of synchronization and the great amplitude of the spikes near the pelvic and tibial ends (Fig. 1, 2). This, together with the fact that most fibres run through the whole length of the muscle (cf. section 5 below) suggests that nearly all fibres are innervated near by, and that the scattered endings are probably additional junctions in the same fibres.

More definite evidence is obtained by comparing the concomitant changes of timing, form, and amplitude of the action potential at different points. An example is shown in Fig. 10. There are 2 main innervation centres, at 11–12 and 26–30 mm, the former being more concentrated, and, therefore, giving rise to a larger and more synchronous spike than the latter. In the intermediate region, the spike is reduced in amplitude and split up into 2 components, (i) an early wave, arising from new myoneural junctions near the recording lead (at 17–19 mm), and (ii) a delayed "hump" due to conducted muscle impulses coming from the main innervation centre. The first group (i) of impulses apparently collides with part of the synchronous volley coming from the main centre, so only a reduced remainder (the delayed hump, ii) is conducted through. Group (i), therefore, belongs to fibres which have 3 (possibly even more) discrete motor nerve endings, while group (ii) has only 2 separate innervation zones.

5. FRACTION OF THE MUSCLE SUPPLIED BY MULTIPLE MOTOR ENDINGS

While the phenomenon of multiple innervation as such was easy to demonstrate, a quantitative estimate of the fraction of the muscle fibres concerned is more difficult to obtain. The very small size, or complete absence, of any "late spike" in the normal action potential (Fig. 1, 2) shows that at least 95 per cent of the fibres which reach through the distance between the 2 main neural zones, *i.e.* through about 60 per cent of the muscle length, have multiple innervation. The problem is, therefore, reduced to the question what fraction of the muscle fibres runs through this intermediate length.

There is evidence that a great majority of the muscle fibres runs through nearly the entire length of the sartorius.

If either end of the completely curarized muscle is stimulated by maximal induction shocks, and the action potential recorded at various distances away from the stimulated region, there is a marked progressive decline in the amplitude of the spike, but little change in its potential-time area (Table 1). This indicates that most fibres run from end to end, and that the progressive decline of spike height during conduction is merely due to different velocities of the impulses in different fibres.

Table 1

Curarized muscle stimulated directly at one end. Position of earth lead at 1/4, 2/4 and 3/4 of muscle length; grid lead at the other end. The potential-time area, in each record, was integrated up to an arbitrary constant interval reckoned from the peak of the spike and chosen so that the diphasic potential change (at the grid lead) did not interfere. Mean values and extreme variations of 5 experiments.

	Recorded at			(4/4) of muscle length.
	1/4	2/4	3/4	
Amplitude	100	75(67-82)	62(48-70)	(61(42-68)*)
Area	100	90(84-94)	92(80-109**)	

* Approximate size of diphasic change at the grid lead.

** This exceptionally large value involves probably a large negative after-potential at this region.

Furthermore, the mechanical twitch tension to a maximum nerve stimulus is only slightly reduced (by 6-12 per cent, mean 10 per cent), if one of the 2 nerve branches is cut. This result may not be very significant, since for various reasons, the mechanical twitch tension cannot safely be considered as a proportional index of the number of active fibres, but it does not disagree with the evidence obtained by electric recording.

In a few cases the fibres of the sartorius muscle were isolated under a low-power binocular microscope, after the frog's leg had been boiled, as described by Buchthal and Lindhard (1939). The great majority of the fibres were found to run from end to end. In one case, out of 100 fibres, only 5 did not reach through the entire length. Short fibres, of only a few mm. length, as described by Mayeda (1890) were not observed. It may be that this discrepancy is due to the different kind of animals used (Buchthal and Lindhard e.g. mention that fibre lengths differ in the sartorius muscles of *Rana esculenta* and *temporaria*), or that the reported short fibres were fragments produced by the dissection.

As a whole, it seems safe to conclude that the great majority of the fibres runs through practically the entire length of the muscle, and that, therefore, nearly the whole muscle is provided with multiple motor nerve endings.

DISCUSSION

The question of a multiple motor nerve supply in skeletal muscle has previously been discussed, but its existence, to any great extent, has not been accepted (see, however, Cattell 1928). According to Fulton (1926) and Wilkinson (1929) multiple motor endings are a rare phenomenon, which indicates that the frog's sartorius muscle must be an exceptional case. Kulchitsky (1924), however, stated that no double motor supply could be found, with methylene-blue staining, in the frog's sartorius muscle, which is in obvious disagreement with the present experiments. It is difficult to see how Kulchitsky arrived at this conclusion since in none of his reported observations did he follow an individual muscle fibre for more than a few millimetres.

Table 1

Curarized muscle stimulated directly at one end. Position of earth lead at 1/4, 2/4 and 3/4 of muscle length, grid lead at the other end. The potential-time area, in each record, was integrated up to an arbitrary constant interval reckoned from the peak of the spike and chosen so that the diphasic potential change (at the grid lead) did not interfere. Mean values and extreme variations of 5 experiments

	Recorded at			
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Amplitude	100	75(67-82)	62(48-70)	(61(42-68)*)
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In view of these statements, we were rather astonished to obtain evidence for a very extensive multiple motor supply in the frog's sartorius, but were no less surprised by a subsequent discovery, *viz.* that corresponding histological evidence had been presented already by Sandmann (1885) in a paper which apparently escaped notice in the more recent literature. Sandmann found, with gold-staining of isolated muscle fibres, that most fibres of the frog's sartorius have 2 or 3 discrete motor nerve endings, some have even more (up to 6), while single endings are only exceptionally observed. He refers also to the presence of distinct "innervation zones."

Sandmann also dealt with the question of "pluri-segmental" innervation of the sartorius muscle. He found that, after severing one of the supplying motor roots, only a few fibres contained a degenerated *and* an intact nerve ending, while most fibres had either completely retained, or lost, their motor supply. More recently, however, Cattell (1928) concluded from a comparison of the mechanical responses with separate and combined stimulation of the motor roots that about $\frac{1}{3}$ of the sartorius (varying between nil and 73 per cent receives "bi-segmental" innervation.

The multiple motor supply may have some functional significance: for as a consequence the impulses need to travel only about $\frac{1}{4}$ of the fibre length to throw the whole muscle fibre into activity, and so external force would be exerted at a somewhat higher rate (see Gasser and Hill, 1924; Hill, 1938). However, the time saved in the spread of the excitation wave is small (about 5 msec. at 25°C), hence it remains doubtful whether much is gained by it.

The peculiar arrangement of the motor endings has, however, a definite experimental interest; it is possible, for instance, to obtain preparation where despite the wide distribution of myoneural junctions, practically the whole of the muscle fibres is innervated in a narrow zone, of a width of one mm, adjoining the nerve-free pelvic end. Such a preparation offers a number of important advantages for the study of neuro-muscular transmission, in particular of local electric potential changes at the myoneural junction. It is intended to report, in a later paper, a series of observations obtained in this way.

SUMMARY

Experimental evidence is described for the existence of multiple motor nerve endings in most fibres of the frog's sartorius muscle.

The electric response of the muscle, to a maximum nerve stimulus, is recorded. In both, pelvic and tibial, regions a large, synchronous spike, of about 50 mV and short latency (1–2 msec.) is usually obtained. This seems incompatible with the facts that the motor nerve endings are distributed over a length of about 2.5 cm. and that most of the sartorius fibres run through the whole length of the muscle (cf. section 5).

The apparent discrepancy is due to the presence of multiple (2 or 3) motor nerve endings on most fibres. Muscle impulses are set up simultaneously at these junctional regions of each fibre; only the impulses which arise nearest to the amplifier leads are recorded, since those more distal are blocked by collision.

This can be demonstrated by various means, *e.g.* by cutting one of the two main nerve branches, by partial curarization, by submaximal nerve stimulation, and by combined application, at suitable time intervals, of 2 stimuli (i) to the motor nerve and (ii) to the nerve-free pelvic end of the muscle. By all these procedures, some myoneural junctions are put out of action and, therefore, some of the "distal" impulses (arising far away) can travel unhindered to the recording leads. In this way, a part or the whole of the normal, synchronous and early, spike potential is abolished and replaced by a discrete potential wave of long latency.

From the variations in size, shape and latency of the spike along the muscle it is often possible to locate 2 or 3 discrete, narrow, "neural" zones, which contain the majority of the myoneural junctions

We are indebted to Dr. J. C. Eccles for helpful discussion and criticism.

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FAST CENTRAL FIBERS IN FISH

PROPERTIES OF MAUTHNER AND MÜLLER FIBERS OF MEDULLOSPINAL SYSTEM¹

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IN TELEOSTS and urodeles two giant nerve cells (cells of Mauthner) are to be found, one on either side of the medulla oblongata at the level of the acoustic decussation (Mayser, 14; Beccari, 3; Bartelmez, 1). In representative teleosts, such as the common catfish of the genus *Ameiurus*, the extraordinarily large sheathed axons of these two cells may be identified through the medulla and most of the spinal cord. They decussate shortly after their origin, and as they pass caudally they give off many short unbranched collaterals which terminate synaptically upon the primary motor neurones of the medulla oblongata and spinal cord (Tagliani, 17; Beccari, 3; Tiegs, 18). The lateral dendrite of each Mauthner cell is exceptionally long and thick and at its terminus is enveloped by a sheaf of synaptic clubs which are the endings of a group of root fibers of the VIIIth cranial nerve (Beccari, 3; Bartelmez, 1; Bartelmez and Hoerr, 2; Bodian, 6). According to Beccari these VIIIth root fibers are of saccular origin.

Bartelmez concluded from his fundamental work on the motor tegmental nucleus of fishes that the Mauthner cells are specialized components of a group of large nerve cells lying in this nucleus and previously homologized by Edinger (8) with the Müller cells of cyclostomes. These cells, of paired paramedian distribution, lie dorsal to the lateral longitudinal fasciculus (*llf*) at the level of the acousticolateral nuclei. They are presumably activatable through intercalary channels originating in the central nuclei of the VIIIth cranial nerve. Their myelinated axons are among the largest in the medulla oblongata, and after decussation pass through the median longitudinal fasciculus (*mlf*) to the ventromedial area of the spinal cord. It is generally believed that the Mauthner cells are differentiated from the rest of this group by short-circuiting of the intercalary channels to them, so that their lateral dendrites receive impulses directly from bipolar acoustic ganglion cells whose peripheral terminals distribute to the saccule of the internal ear.

The present investigation deals with the properties of the fibers passing from the *mlf* of the medulla through the spinal cord. Knowledge of their properties is a necessary prelude to the study of their synaptic activation.

¹ This work was aided by a grant from the National Research Council to the Department of Pharmacology. A preliminary report appeared in *Amer. J. Physiol.* 1940, 129: 433.

METHOD

In adult catfish (*Ameiurus melas* and *nebulosa*), the potentials propagated down the spinal cord following stimulation of the *mlf* in the medulla oblongata were recorded by means of a differential amplifier and a cathode ray oscillograph (mean length of cords, 50–115 mm.). Each fish was kept in water at 10–15°C

24 hours before, and during the experiment was aerated. The water was changed every 24 hours. The fish were kept in a container which permitted aeration could occur but no water could enter the container. The fish were anesthetized by mited to opening the cranial cavity and exposing the vertebral column. At the temperatures used, minimal amounts of chlorotone dissolved in the water provided sufficient anesthesia. Respiration usually continued to the end of the experiment.

Steel needles insulated to their tips (16–100 μ in diameter) were used for both recording and stimulating. Paired stimulating electrodes (1.5–2 mm. apart) were inserted vertically into the medulla on either side of the *mlf* to a depth decided upon from previously deter-

mined points in the cord 4–5 mm. caudal to the first, or in the vertebral cartilage 10–20 mm. caudal. The timing of the oscillograph trace sweep with the discharge of the stimulating thyatron was controlled electrically (Loeffel, 13). As far as possible, stimulation was confined to a range which was subthreshold for muscle potentials, when muscle potentials were present they were recorded separately from the cord fiber potentials by means of electrodes inserted into the muscle near the recording electrodes in the cord. As all muscle potentials occurred after a delay of at least 5 msec. following the stimulus, they were readily distinguished from the prompt cord fiber potentials in the records from the cord.

Several procedures were used in securing histological controls upon the experimental studies. In accordance with the suggestions of Bartelmez (1), Bartelmez and Hoerr (2), and Bodian (6), from several fish perfused with chilled formol chrome sublimate, the medulla and spinal cord were removed rapidly, mordanted in potassium dichromate, and sectioned at 5 μ . The sections of this material, stained with Azan Mallory, are designated as "cytologically prepared." Myelin sheaths were stained by the Kultschitzky method applied upon Weigert mordanted material, or by 2 per cent osmic acid. For axon preparations the "short protargol" method of Davenport, McArthur and Bruesch (7) was followed. The traces of recording or stimulating electrodes were examined in serial 20 μ sections stained in cresyl violet.

HISTOLOGICAL OBSERVATIONS

1. *Anatomical relations of the median longitudinal fasciculus.* Figure 1 illustrates the fiber composition of the medulla oblongata of *Ameiurus* at the most rostral level of stimulation. Thus, hereafter designated as the "rostral level," corresponds to line 1 of the inset figure, an outline sketch of a dorsal view of the medulla and cerebellum adapted from Bartelmez (1). Figure 1 is a photomicrograph from a 5 μ transverse Weigert series through the brain of an *Ameiurus* of 66 mm. cord length. The central rectangle is located within the densest aggregation of large myelinated fibers in the medulla. These fibers are coursing longitudinally and the aggregation includes the *mlf* and the *llf*. The rectangle is set asymmetrically so that it divides the *mlf* from the *llf* on the side toward the inset figure and includes the medial part of the *llf* on the side away from it. At the level of line 1 the *mlf* is divided into supra-(sc) and infracommissural (ic) bundles by the acoustic commissure (ac). The arrow under *D* (dorsal) in the figure indicates the position of the two Mauthner fibers in the sc bundle. These fibers originated from the Mauthner cells a few sections rostrally and decussated a few sections caudally.

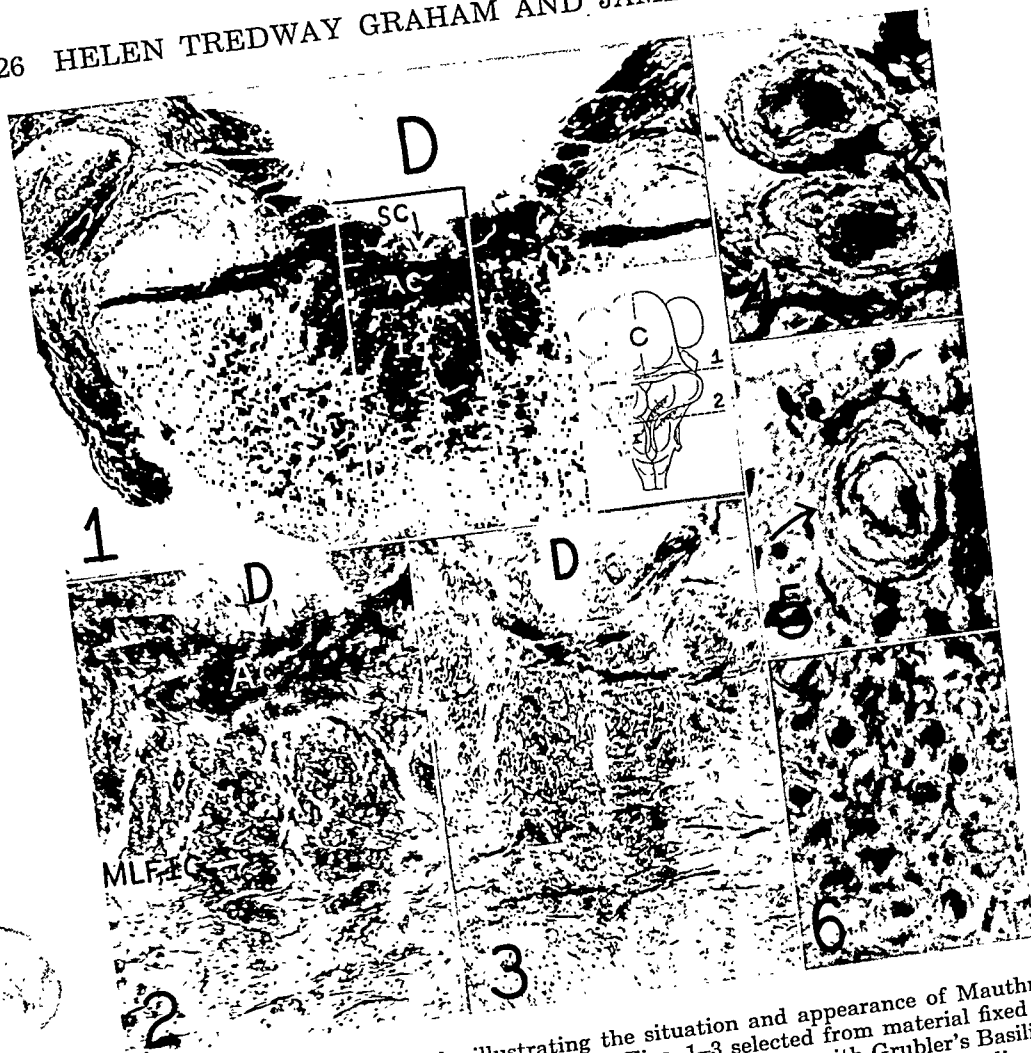


FIG. 1-6. Photomicrographs illustrating the situation and appearance of Mauthner and Müller fibers in the medulla of *Ameiurus*. Figs. 1-3 selected from material fixed by perfusion in formol bichromate, Weigert mordanted, and colored with Grubler's Basiline. Figs. 4, 5 and 6 selected from a medulla fixed by perfusion with formol chrome sublimate; sections colored with Azan Mallory.

FIG. 1. ($\times 29$) Cross-section of the medulla at the "rostral level." The median (*mlf*) and a portion of the left lateral longitudinal fasciculus are enclosed within the rectangle. The two Mauthner fibers (arrow) are situated in the supracommissural (*sc*) bundle just dorsal (*D*) to the acoustic commissure. The inset is an outline sketch of the medulla and cerebellum of *Ameiurus* viewed from its dorsal surface. *C*, cerebellum; *VII*, lob. *VII*; *X*, lob. *X*. Line 1, "rostral level" of stimulation illustrated in Figs. 1 and 2; line 2, "caudal level" of stimulation, Fig. 3.

FIG. 2. An enlarged view ($\times 58$) of the area contained within the rectangle of Fig. 1. The acoustic commissure (*ac*) divides the *mlf* into supra- and infra-commissural bundles. Note that the large myelinated fibers of the *mlf* do not extend to the ventral midline of the medulla.

FIG. 3. View ($\times 58$) of the same area as Fig. 2 but corresponding to level 2 of the inset figure.

FIG. 4, 5 and 6 ($\times 580$). Fig. 4. Transverse section of the decussation of right and left Mauthner fibers. Note the relative thickness of myelin sheath and axon, lack of homogeneity in the sheath and dissymmetry in size of the two axons. Fig. 5 is a section 90μ caudal to that of Fig. 4; the axon of the Mauthner fiber indicated by the arrow has expanded and stains less deeply. Fig. 6 from same section as Fig. 5, illustrates a typical fiber area of *mlf*, *ic*.

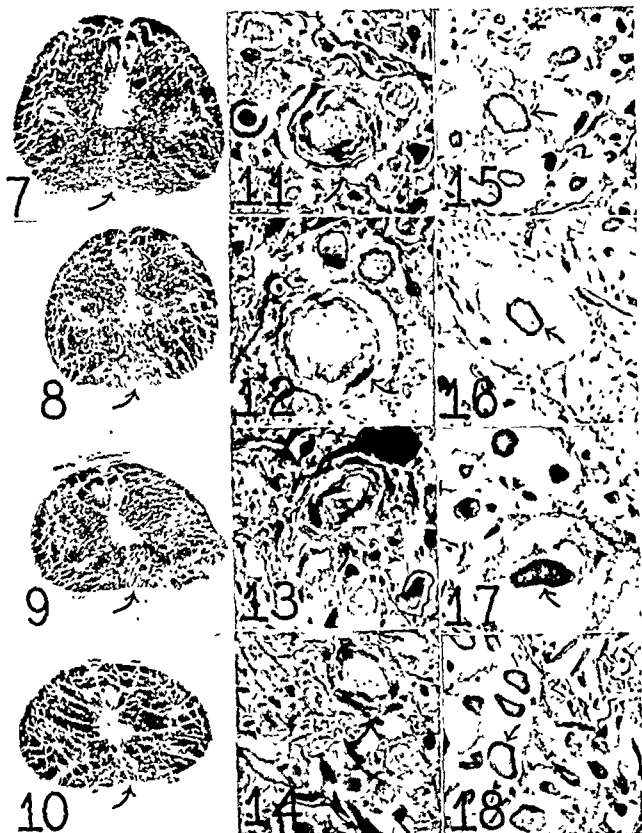


FIG. 7-10 ($\times 35$). Low-power orientation series of the spinal cord of *Ameiurus*, representing levels 60, 75, 90 and 115 mm. caudal to the origin of the Mauthner fibers. Cord length 140 mm. Arrows indicate the situation of the ventromedial fiber bundle. Formol bichromate fixation, Weigert mordanted, Kultschitzky stain.

FIG. 11-14 ($\times 580$) allow comparison of a single Mauthner axon and its sheath at cord levels comparable to those illustrated in Fig. 7-10. In each figure arrow ends at the outer margin of the myelin sheath. In Fig. 11 and 12 medium-sized Muller fibers (11 μ) are included for comparison. Cord length 66 mm., formol chrome sublimate perfusion, Azan Mallory stain.

FIG. 15-18 ($\times 580$). Mauthner axon (arrows) in silver preparations; levels 9, 35, 71 and 115 mm. caudal to the origin of the Mauthner fiber. Note that at the 115 mm. level this Mauthner axon was no larger than an adjacent Muller axon. Cord length 140 mm.; "two-hour" protargol method.

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Table 1. *Diameters of Mauthner and Müller fibers. Average outside diameters in microns of two Mauthner and ten largest Müller fibers. Total length of cord about 140 mm. in all cases except the first two where it was 66 mm.; in these two cases the cord level measurements were corrected by the appropriate factor.*

Control Series							
Date and Method	Class of Fiber	Medulla	Cord level corresponding to conduction distance of:				
			30 mm.	60 mm.	75 mm.	90 mm.	115 mm.
11-1-39 W	Mauthner Müller	32.0 15.8		43.0 22.4	30.0 20.1		26.0 18.7
11-2-39 FSA	Mauthner Müller			32.5 17.3		29.5 16.0	27.0 13.5
6-8-40 W	Mauthner Müller			38.0 21.3	34.0 18.7	29.0 14.6	28.5 14.5
7-10-40 FSA	Mauthner Müller	39.0					
7-10-40 FSA	Mauthner Müller	33.0					
7-10-40 FSA	Mauthner Müller	32.0					
7-10-40 FSA	Mauthner Müller	40.0					
7-15-40 W	Mauthner Müller			36.0 13.0		** 10.6	
7-15-40 W	Mauthner Müller			33.0* 18.1		** 14.2	
7-15-40 W	Mauthner Müller			28.0* 13.5			
7-15-40 W	Mauthner Müller			29.5 13.9		28.5 11.1	
Experimental Series							
5-8-40 W	Mauthner Müller	39.5 16.3		41.0 16.3		22.0 12.8	
5-16-40 FO	Mauthner Müller		38.0 20.2	38.0 19.6		28.0 15.3	45.0 17.7
5-20-40 FO	Mauthner Müller			30.2 16.1	** 12.7		
6-1-40 FO	Mauthner Müller					** 19.4	

Table 1—Continued
Experimental Series—Continued

Date and Method	Class of Fiber	Medulla	Cord level corresponding to conduction distance of:				
			30 mm	60 mm	75 mm.	90 mm.	115 mm.
6-25-40 FO	Mauthner Muller		39 0 16 7	36 0 17 6		31 5 15 7	26 5 11 8
7-8-40 O	Mauthner Muller			33 5 19 6	26 0* 20 8		
7-9-40 O	Mauthner Muller				29 5 19 7		26 0 19.3
7-9-40 O	Mauthner Muller			36 0 22 5		43 0 21 4	

FSA—fixation by perfusion of chilled formol chrome sublimate, processed by accepted cytological procedure; Azan Mallory stain

W—fixation by immersion in formal bichromate mordanted in Weigert, stained by Kultschitzky's method

O—fixation by immersion in 2 per cent osmic acid.

FO—fixation in aqueous 10 per cent formol, stained in the block by 2 per cent osmic acid

* Only one Mauthner fiber identified.

** Neither Mauthner fiber identified.

In Fig. 2 the area enclosed in the rectangle of Fig. 1 has been enlarged. Examined in conjunction with Fig. 1 it illustrates that the densely packed large myelinated fibers of the *mlf* do not extend to the ventral midline of the medulla and that elsewhere than in the *mlf* and *llf* the myelinated fibers in the medulla are not disposed in large longitudinally coursing bundles. Figure 3 shows the *mlf* at the level (hereafter designated as the "caudal level") of line 2 of the inset figure. The relations of the *mlf* and *llf* have not changed significantly from the "rostral level," though the commissure is much diminished.

2. *Course, size and histological characteristics of Mauthner fibers at medullary and cord levels.* Following their decussation at the level of the acoustic commissure (Fig. 1 and 2) the two Mauthner fibers continue caudally in the superficial zone of the *mlf* (Fig. 3) and enter the ventromedial area of the spinal cord where they come to lie just ventral to the central gray (Fig. 7-10). Both Mauthner fibers could be identified as far as 90 mm. caudal to the level of the acoustic commissure in most of the fish, and even at 115 mm. it was frequently possible to distinguish them from the Müller fibers (see page 227) by their greater size and sheath thickness.

With appropriate care in the orientation of blocks of spinal cord for embedding, the outside sheath diameter of the same Mauthner fiber may be measured in the medulla and at various cord levels. In Table 1 the average diameters of the two Mauthner fibers are presented.

The members of each pair of the 6 measured at the medullary level were of nearly equal size, and varied between the limits of 32 and 40 μ . Usually the Mauthner fibers are not significantly reduced in outside diameter for the first 60 mm. of their course through the spinal cord (140 mm. cord length); but farther down in the cord the size may be diminished so that a fiber measuring 40 μ at 60 mm. may measure 25 μ at 90 mm. (Table 1). Local fluctuations in diameter also occur, and these may be much accentuated in cords fixed in formalin and later osmicated. In the single Mauthner fiber selected for the photomicrographs of Fig. 11 to 14, the limits of chance fluctuation in fiber diameter as well as axon-sheath ratio are apparent by comparison of Fig. 11 and 12. Figures 13 and 14 at the same magnification show the reduced diameter which usually characterizes the caudal extremity of the Mauthner fiber.

The sheath of the Mauthner fiber reduces osmic acid, stains blue black by the Weigert method, and is soluble in fat solvents. In cytological preparations (formol chrome sublimate-Azan Mallory) the myelin sheath and axon color differentially red and blue respectively (Fig. 4 and 5 and 11-14). The red matrix of the myelin sheath is latticed by concentrically disposed slender blue fibrillae, which to our knowledge have not been previously described. Typical nodes of Ranvier were not observed, but node-like reductions in the diameters of the axons occur; these are accompanied by dense staining. This is evident by comparison of Fig. 4 and 5 which illustrate closely adjoining cross-sections of the same fiber. The axoplasm appears homogeneous save for scattered mitochondria; an axolemma was not distinguished and there was no separation into cortical and medullary zones (see also Fig. 15-18, "short protargol" method).

The axon as well as the outside diameter of each of the two Mauthner fibers has been measured in four medullas prepared cytologically. Since there was no retraction of the axon from the encasing sheath in any of these, the measurements can be considered accurate within the limits of shrinkage incurred during fixation. The eight fibers thus measured had an average axon diameter of 18.5 μ , outside diameter of 37.2 μ , the ratio of the former to the latter being 0.5. Four spinal levels of a single Mauthner fiber were similarly measured. These corresponded to the usual 60, 75, 90 and 115 mm. conduction distances used in the experimental series. The ratios of axon to outside diameters in μ were respectively

16.0	24.0	14.0	13.0
27.5'	30.0'	18.5'	23.0'

There was no evidence to suggest that these ratios were significantly different in smaller and larger fish (66 and 140 mm. total cord length).

3. *Myelinated fibers which enter spinal cord from median longitudinal fasciculus.* The giant Mauthner fibers can be traced individually through the spinal cord, but there is no direct anatomical evidence bearing upon the number of other large myelinated fibers which pass from the *mlf* into the ventromedial region of the cord. Such evidence could only be obtained by

Marchi degeneration following medullary lesions, or retrograde degeneration occurring subsequent to division of the spinal cord, and these procedures have not been carried out. However, since the continuity of the *mlf* with the ventromedial cord area is apparent in serial histological sections through the medullospinal junction, that area certainly should contain a high percentage of fibers derived from the *mlf*.

Figures 7 to 10 are low power photomicrographs of 5 μ Weigert sections which illustrate the relative size of the cord and its contained gray matter at distances 60, 75, 90 and 115 mm. caudal to the level of the acoustic commissure. The ventromedial fiber bundle (arrows) is most apparent in Fig. 7 and 8 (60 and 75 mm. respectively). Here the close aggregation of thin sheathed fibers of large size has caused the area to stain less intensely than the remainder of the white matter. In Fig. 9 and 10 (90 and 115 mm. respectively) the larger myelinated fibers of the bundle, with the exception of the Mauthner fibers, have approached the ventral periphery of the cord. The zone immediately surrounding the Mauthner fibers, which lies beneath the central gray, is made up chiefly of smaller myelinated and non-myelinated axons. For convenience we shall refer to the large thin-sheathed medullospinal axons of the ventromedial bundle as Müller fibers.

The sheaths of the Müller fibers are similar in staining properties and solubility to those of the Mauthner fibers, but they do not exhibit evidence of concentric lamination of the myelin matrix. Figure 6 is a cross-section of a group of the fibers of the *mlf*, *ic* bundle near the 'rostral level.' The Müller fibers at this level are scattered among numerous smaller ones so that no fair comparison may be made between average size in the *mlf* and in the ventromedial bundle of the spinal cord.

The measurements of the Müller fibers at different cord levels have been abridged to the average outside diameters of the 10 largest fibers (Table 1). In measurements of the diameters of at least 100 fibers selected at random from the ventromedial bundle at each cord level recorded in the table, the fibers were found to grade in size from the high value published to a minimum of 2 to 4 μ ; non-myelinated fibers also occur. From Table 1 it is evident that the Müller fibers are somewhat reduced in diameter between the 60 and 115 mm. levels. In one specimen (6/25/40) an average reduction of 5.8 μ occurred, in another (5/16/40) the average reduction in diameter was only 1.9 μ .

In four cord levels of one preparation, measurements in μ of both the axon and the outside diameters of the 10 largest Müller fibers were averaged. These levels correspond to the usual conduction distances of 60, 75, 90 and 115 mm., and the ratios are directly comparable to those given for the single Mauthner fiber measured in the same sections (page 230). The ratios for the

Müller fibers were respectively $\frac{15.2}{19.6}$, $\frac{14.0}{19.2}$, $\frac{12.2}{16.6}$, $\frac{12.0}{17.2}$ at the 4 levels.

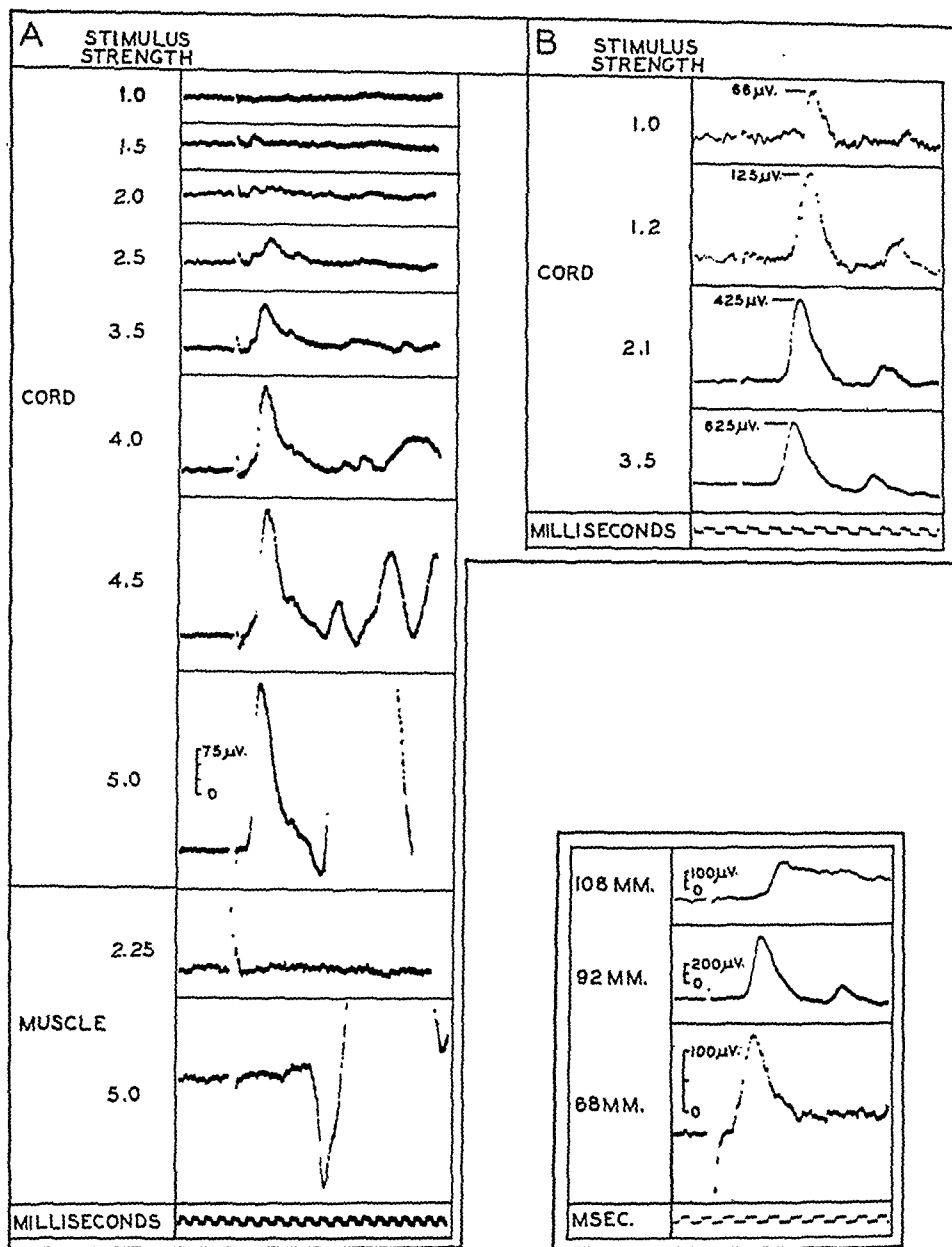


FIG. 19

FIG. 20

FIG. 19. Cord and muscle responses to stimuli of different strengths. Relative strength of stimulation indicated (weakest used in each case = 1).

A. Pair of stimulating electrodes 0.5 mm. behind "rostral level." First 8 records: two recording electrodes in cord, more rostral one 52 mm. from stimulating electrodes. Last 2 records: recording electrodes on muscle, 5 mm. from position of electrodes in cord. All records of series at same amplification. Temperature 13.5°C . 4/18/40.

B. Pair of stimulating electrodes 0.5 mm. in front of "caudal level." Single recording electrode in cord, 92 mm. from stimulating electrodes. Amplification decreased for records with greatest strengths of stimulation. Temperature, 12.5°C . 6/3/40.

FIG. 20. Responses at 3 conduction distances. Same preparation as for Fig. 19B; stimulus maximal. Conduction rate, 50-56 m./sec.

EXPERIMENTAL OBSERVATIONS

1. *Continuous activity in cord.* Early in some experiments, the cord showed rhythmic activity consisting of waves of several hundred microvolts 0.6–1.4 sec. apart. When present, this activity was at times increased in amplitude though not in frequency by stimulation of the saccular otolith, or occasionally it was induced by such stimulation. It seems to be similar to the rhythmic activity observed by Shurrager (16) in brains recently isolated from catfish, and to like potentials recorded from the central nervous systems of many animals by other workers. In addition the catfish cords showed a persistent, continuous activity consisting of irregular oscillations of a few microvolts, clearly visible in all records taken with sufficient amplification. This background activity interfered only with the smallest waves induced by stimulation, and no effort was made to control it.

2. *Form of response in cord; conduction time.* Electrical stimulation of the medulla results in the appearance in the cord of an electrical disturbance which is recorded as a complex of waves, chiefly negative in sign. If the stimulating electrodes are inserted vertically at the "rostral level" so that they are in the region of the Mauthner-Müller cells, or if they are inserted behind this level, the response will usually appear in the cord within several msec., and the exact time interval will be such that the rate of the fastest impulses (calculated from conduction time and distance without allowing for the latent period) is 40–60 m./sec. The conduction time between these medullary levels and the cord is the same whether the impulse is transmitted away from or towards the medulla. It may be concluded that below the acoustic commissure there are no synapses in the pathway of the fast impulses. If the stimulating electrodes are placed rostral to the commissure, the conduction time will be significantly (1 msec. or more) longer than at lower levels; evidently synapses in the pathway of the fast impulses occur immediately in front of the "rostral level," but the situation has not yet been investigated in detail electrically.

The cord response to maximal medullary stimulation at or behind the "rostral level" (Fig. 19 and 20) consists mainly of a relatively large single negative wave lasting 1–3 msec. (maximum amplitude typically 100–500 μ V.). This main wave may be preceded by a much smaller wave of 5–20 μ V. lasting 0.5 msec. or more, and may be followed by other small waves for an interval of 5 msec. or more. The conduction rate for the main wave varies from the figure given above for the fastest impulses (40–60 m./sec.) to 10–20 m./sec. for the slowest ones, while the rate for the small initial wave when present is never less than 50 m./sec. and never significantly greater than the fastest rates observed for the main wave in the absence of the initial wave.

The fastest conduction rate as determined at various distances tends to be the same in each preparation (Fig. 20), and the recorded form is not markedly affected by temporal dispersion except sometimes at conduction distances greater than 100 mm. (cf. Fig. 20, records at 92 and 108 mm.;

Fig. 22). Finely-measured changes in depth of the recording electrode at any one level in the cord alter only the magnitude of the response, not its time relations or recorded form. The form can however be modified by changing the strength of stimulation or the locus of stimulation in the medulla.

The effect of strength of stimulation on the recorded form is illustrated in Fig. 19 and 21. The preparation used for Fig. 19A contained a few fast

fibers (62 m./sec.) which produced a small wave with low strengths of stimulation. A later wave, formed by the response of the numerous fibers with conduction rates of 35 down to perhaps 20 m./sec., came in gradually with stronger stimulation, while with strong enough stimulation, the potential was recorded as a single continuous wave. The preparation used for Fig. 19B gave no evidence of fibers with faster rates than about 50 m./sec., and a doubtful initial wave with weak stimulation only.

With near-threshold strengths of stimulation it was usually possible, especially if the stimulus was applied at the "rostral level" (see below), to obtain records consisting of a succession of small, discrete waves, indicating groups of fibers with different conduction rates more or less equally accessible to the stimulus. With stimulation sufficiently near threshold, some of these small waves have the characteristics of single fiber responses, though more of them probably represent the activity of homogeneous groups of very few fibers. A change of less than

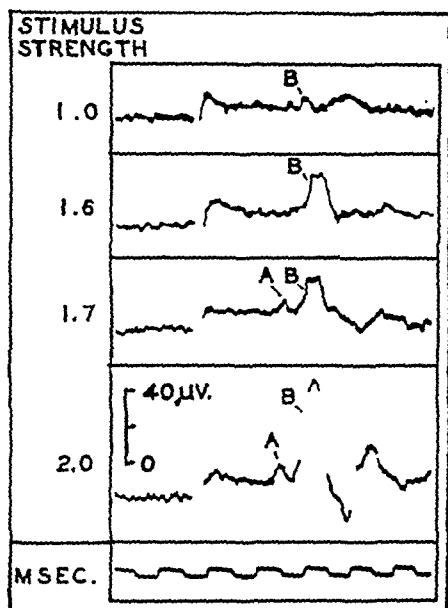


FIG. 21. Single fiber responses. Monopolar stimulation at "rostral level." Relative strength of stimulation indicated. One recording electrode in cord, 96 mm. from stimulating electrodes. Conduction rates: wave A, 60 m./sec.; wave B, 48 m./sec.; start of slower wave in first record, 37 m./sec. Temperature 15.5°C. 7/9/40.

1 per cent in stimulus strength may suffice to bring in or leave out one of these groups; that they actually are groups and not single fibers is indicated by the longer duration and higher voltage of their responses than of the true single fiber responses recorded, and also by their splitting up into smaller waves with rapidly repeated stimulation (not illustrated). Apparently the fibers composing the groups differ more in their recovery characteristics than in other properties.

In the records reproduced in Fig. 21, the lowest strength of stimulation produces a small, discrete wave (B) interpreted as indicating the activity of

a single fiber with a conduction rate of 48 m./sec., and a longer later wave which probably results from the activity of several fibers with slightly different conduction rates in the neighborhood of 35 m./sec. An increase of 63 per cent in stimulus strength does not increase the potential recorded from these slow fibers, but more than doubles the height of wave B. The cord evidently contained a number of excitable fibers conducting at about 48 m./sec. A further slight increase (4 per cent) in the stimulus makes no change in wave B, but introduces a preceding small wave (A, conduction rate 60 m./sec.), the original height of which is not more than doubled by further increase in stimulus strength.

Waves A and B resemble both in magnitude and duration the smallest of the initial waves described above as preceding the large main response. It may be concluded that such waves at their smallest result from the activity of single fibers, but because they do not sufficiently exceed in size the waves of continuous background activity, it cannot be concluded that they accurately reproduce the true form of the individual spike. Definite statements regarding this form must await more selective recording of the potential; the apparent duration (0.4–0.5 msec.) in the available records is probably too short. However, it is worth noting that the form seems to be the same for all the single fibers observed, with conduction rates from 60 down to 15 m./sec.

The importance of the position of the stimulating electrodes in the medulla is illustrated in Fig. 22. The records of series I were obtained from the caudal portion of the cord following stimulation of the medulla at the "rostral level," those of series II from the same position in the cord following medullary stimulation at the "caudal level." Comparison of the two series as wholes brings out the greater tendency to separation of the waves and the lower maximum potential (about half that in series II) with stimulation at the "rostral level" (cf. the discrete potential waves and the similar stimulating position of Fig. 21). In each series, the response of the fast fibers is very small at the most dorsal effective stimulating depth, but increases rapidly with increasing depth of the electrodes; within the limits of error of location of the stimulating electrodes, the maximum responses in each series is obtained with stimulation in the region of the *mlf*. Stimulation is however still effective, particularly on the slower fibers at the "rostral level," when it is applied well below the *mlf*, even in the fluid below the medulla.

3. *Effect of temperature on conduction rate.* The results described above were obtained at temperatures varying from 10° to 15°C. for the whole group of experiments, but constant within about a degree for any one experiment. In a few experiments, records were made at several distinct temperatures; data obtained on the conduction rate of the fastest fibers in these experiments are summarized in Table 2. The temperature coefficient (Q_{10}) calculated from these data varies from 1.3 to 1.9 for temperatures ranging from 23° to 8°C., the higher values falling in the lower temperature ranges.

4. *Recovery cycle.* Observations upon the recovery cycle of the fast fibers

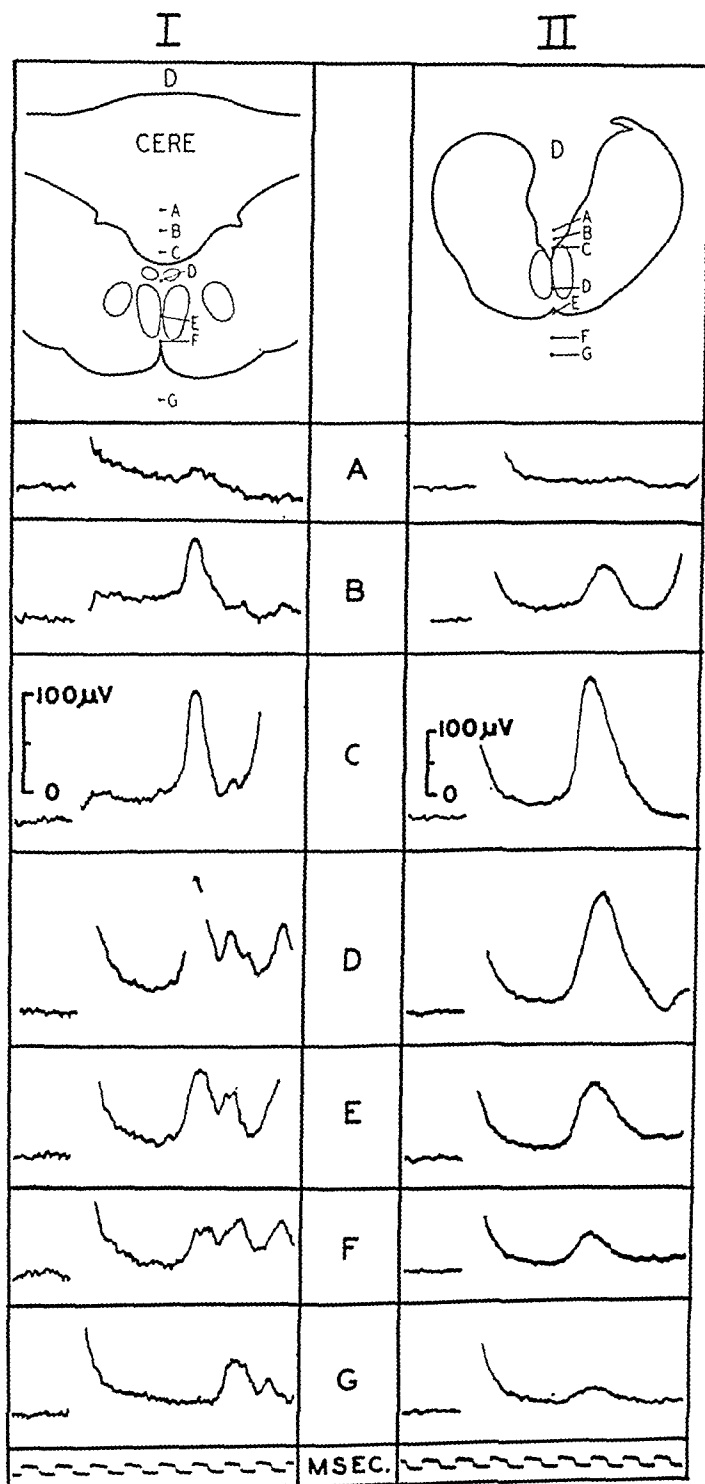


FIG. 22. See next page for legend.

in the cord following stimulation in the medulla are interfered with by the presence of slow pathways, activated by shocks of the strength required for the observations in question, and apparently more readily activated by two successive shocks than by a single one. Nevertheless, sufficiently accurate data have been obtained with medullary stimulation through a single pair of

Table 2. Temperature coefficient of conduction rate. Stimulating electrodes in medulla between "rostral" and "caudal levels"; recording electrodes on cord.

Date	Temperature °C.	Conduction distance mm.	Conduction rate m./sec.	Temp. coef. Q_{10}
2/7/40	16	58	58	
	23		73	1.4
	15		58	1.3
	11.5		48	1.5
	8		39	1.9
4/20/40	13.5	67	42	
	21.5		51	1.3
5/9/40	11.5	61	35	
	17.5		51	1.9
	22		61	1.5

electrodes for conditioning and testing shocks to justify the following general statements about the recovery cycle of the fast fibers. The absolutely refractory period is of the order of 1 msec., while the relatively refractory period varies from 2 to 10 msec. During the relatively refractory period, the rate of conduction is slowed, as in peripheral nerve. This period is usually followed by a period of supernormal excitability which lasts several hundred milliseconds; the maximum excitability (greatest observed, 10 per cent above the resting excitability) falls 10-20 msec. after the conditioning shock. The

FIG. 22. Effect of changing position of stimulating electrodes. Pair of paramedian stimulating electrodes, 10-115 mm. below stimulating electrodes. Amplitude of shocks, 10-115 mm. below stimulating electrodes. Amplitude of shocks, greater in I than in II, as indicated by calibrations in records C. Temperature, 10.5°C. 6/25/40.

I. Stimulating electrodes at "rostral level"; the lettered dots in drawing I indicate the approximate positions of the electrodes when stimulation produced the records similarly lettered in series I. The ovoid areas next to the midline in the drawing include the area enclosed within the rectangle of Fig. 1.

II. Stimulating electrodes 0.5 mm. in front of "caudal level"; lettered dots of drawing II and series II records related as in I.

existence of a subnormal period following the supernormal period has not been observed following a single shock, but considerable subnormality is developed by repetitive stimulation, even at low frequencies.

DISCUSSION

The above description of the properties of catfish central fibers clearly shows their close resemblance to amphibian peripheral fibers of the A group. The catfish fibers are larger than frog A fibers in general, but the outside diameter of the Müller fibers is not significantly greater than the 20μ reported for some bullfrog A fibers by Erlanger and Gasser (9). The axon diameter of these two sets of fibers is probably also about the same: if the value 0.75 holds for the ratio $\frac{\text{axon diameter}}{\text{outside diameter}}$ of the bullfrog fibers as of large A fibers of the green frog (Schmitt and Bear, 15), the axon diameter of the large bullfrog fibers is about 15μ , actually greater than the measured axon diameters of the Müller fibers (average value, 13.4μ). The Mauthner fibers are of course somewhat larger, even in the cord and even with respect to axon diameter; in the medulla and with respect to outside diameter they are notably larger than the frog fibers.

In estimating the resemblance in physiological properties, allowance must be made for the (roughly) ten-degree difference in temperature between most of the experiments on catfish and those on frogs. At $20-25^{\circ}\text{C}$., frog fibers conduct up to 55 m./sec. (Erlanger and Gasser, 9), their absolutely refractory period is as short as 0.8 msec. (Bishop and Heinbecker, 4), their relatively refractory period 1.5 msec. or longer (Graham, 12). When appropriate allowance is made for temperature, the catfish fibers seem actually to conduct more rapidly than frog A fibers—a state of affairs consonant with their larger size—and to recover no less rapidly.

Other points of similarity between the two sets of fibers are the marked supernormal phase of the recovery cycle and the temperature coefficient of the conduction rates. The temperature coefficient is similar in magnitude as well as in being greater in lower temperature ranges (Gasser, 10). The only possible significant difference between the two sets of fibers is in the time-relations of the spike, since the spike rising time of frog A fibers is not less than 0.3 msec. (Blair and Erlanger, 5) at room temperature, while the apparent duration of the entire spike of the catfish fibers even at the lower temperature of these experiments does not greatly exceed this time interval. But the uncertainty involved in this observation on fibers in tissue with continuous background activity precludes attributing significance to this difference between the two sets of fibers in view of their similarity in all other respects.

The source of the responses from the catfish cord is believed to be the larger fibers of the ventromedial bundle for the following reasons. (i) The response is maximal when the stimulating electrodes are placed in the region of the *mlf* in the medulla (Fig. 22), from which the ventromedial fibers of the

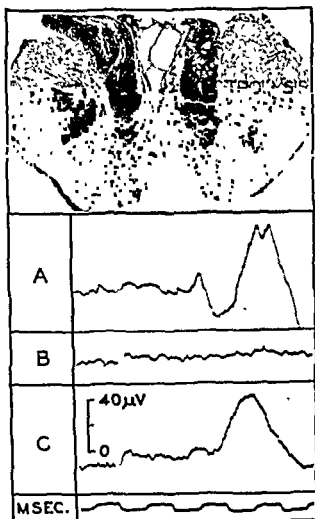


FIG. 23

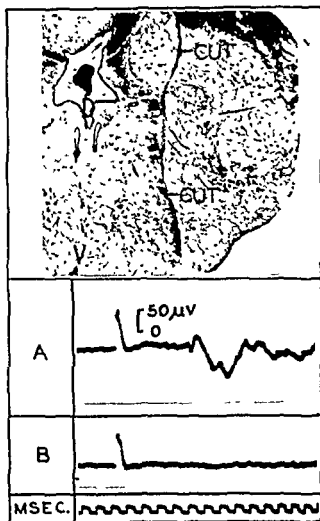


FIG. 24

FIG. 23. Effect of electrolytic destruction of midline of medulla. Stimulating electrodes in cord, 94 mm. from first position of recording electrode. Recording micro electrode in medulla; first position, "caudal level," records A and B; second position 4 mm. behind "caudal level," record C. After record A taken, electrolysis with 15 mA. for 30 seconds at first position; records B and C then taken. The response is completely eliminated at the region electrolyzed (record B), but still present in the ventromedial fibers caudal to this region (record C). Photomicrograph: cross-section (colored with haematoxylin and eosin) near caudal end of lesion shows its dorso-ventral and lateral extent; rostro-caudal extent of lesion 4 mm.; no visible evidence of lesion at second position. V = ventral midline of medulla. Temperature, 10°C. 7/1/40.

FIG. 24. Cord response to stimulation of the sacculus before (record A) and after (record B) separation of the incoming paths of the ipsilateral VIIIth nerve from the *mlf*. Pair of stimulating electrodes placed in contact with saccular otolith; recording electrodes in cord 70 mm. away. Photomicrograph: cross-section (colored with cresyl violet) of ipsilateral half of medulla showing cut at "rostral level," about 2 mm. in rostro-caudal length, through the acoustic commissure and juxtaposed neuropil. Temperature, 13.5°C. V = ventral midline of medulla. 2/9/40.

cord come; when the stimulating electrodes are moved laterally in the medulla the response disappears unless the strength of stimulation is increased.

(ii) The response to stimulation at the "caudal level" is greater and less scattered but no faster than the response to stimulation at the "rostral level" (Fig. 22). This is in agreement with the histological relationships, since those fibers of the ventromedial bundle that arise from cells lying behind the "rostral level" would be stimulated from electrodes at the "caudal level"

but not at the "rostral level"; these fibers do not differ in size (therefore presumably not in conduction rate) from those arising more rostrally. (iii) Stimulation of the cord produces responses recorded from the medulla with the same conduction rate as impulses propagated in the reverse direction, and these responses can be eliminated by electrolysis of the *mlf* (see Fig. 23 and legend). (iv) Cord responses which are believed to occur in the same fibers as those from medullary stimulation can be produced by stimulation of the saccule; their time of appearance in the cord is of course later by the period of at least one synaptic delay. Description of these responses is not included in this report, but Fig. 24 gives an examples of them, and the effect of severing the endings of the nervous pathways from the saccule to the *mlf*. This experiment leaves no room for doubt that the cord responses to saccular stimulation occur in the fibers of the *mlf*. (v) The conduction rates of the responses to medullary stimulation indicate that they must occur in large fibers, and most of the large fibers of the cord are in the ventromedial bundle. Moreover, the similarity of the ventromedial fibers to frog fibers in size, and of the catfish cord responses to frog A responses, indicates the correctness of this view.

While histological study of the individual cords from which records were made has not explained on the basis of fiber size the variations from experiment to experiment of either the form as recorded or the fastest conduction rate observed, the occurrence in many experiments of a small initial wave appearing with relatively weak stimulation and not increasing greatly in size with stronger stimulation, indicates the presence in the cord of a couple of fibers set off from a much more numerous group by their conduction rate. The conduction rate of the start of the main wave following such a small initial wave is roughly 0.8 of the rate of the small wave (see the typical relationship in Fig. 21). This ratio is much greater than the ratio between the average outside diameters of the largest Müller fibers and of the Mauthner fibers (cf. Table 1) but is about the same as the ratio between the average axon diameters of these fibers (0.75–0.8 as calculated from the data on pages 229 and 230). Therefore, on the hypothesis of direct proportionality between axon diameter and conduction rate (Gasser and Grundfest, 11), the assignment of the initial and main waves to the Mauthner and Müller fibers respectively seems warranted.

SUMMARY

1. The spike potentials which appear in the spinal cord following electrical stimulation of the *Ameiurus* medulla resemble amphibian A fiber responses in conduction rate, recovery cycle, and temperature coefficient of conduction rate.

2. Since the fastest of these potentials travel at the rate of 50–60 m./sec. at 10–15°C., the fibers producing them must be larger than frog A fibers, if the usual theory relating fiber size and velocity applies.

3. Fibers of sufficient size to account for the cord spike potentials pass

into the ventromedial bundle of the spinal cord from the median longitudinal fasciculus of the medulla, which contains the axons of the Mauthner and Muller cells.

4. The outside diameters of the Mauthner fibers vary between 43 and 22μ in the cord and medulla, while the axon diameters vary between 24 and 13μ . The heavy myelin sheath accounts for about half of the total fiber diameter in the medulla; it becomes progressively thinner down the cord, without however becoming as thin in relation to fiber size as the sheaths of the other large fibers. The matrix of the Mauthner fiber sheath colors red in Azan Mallory, and is latticed by concentrically disposed, blue-staining fibrils.

5. The average outside diameters of the ten next largest fibers (Muller fibers) vary between 22.5 and 11.1μ at different levels and in different preparations. The average axon diameter is about 0.7 of this. There is no constant difference in relative sheath thickness at different levels, and the sheaths appear homogeneous.

6. In correlation with the diameter measurements of the Mauthner and large Muller fibers, the properties of the fast cord spike potentials indicate that they arise in these fibers. The effects on the response of changing the location of the stimulus in the medulla and the absence of other elements in the cord likely to produce such a response, point to the same conclusion.

7. Stimulation of the saccular otolith causes responses in the cord which apparently occur in the same fibers as the responses to medullary stimulation; the responses to saccular stimulation have been shown to pass through the median longitudinal fasciculus.

8. Responses of single fibers, of various conduction rates from 60 to 15 m./sec., may be recorded from microelectrodes in the cord, particularly if the stimulus is placed rostrally in the medulla. The single fiber responses all appear to have the same spike form.

Grateful acknowledgement is made to the United States Bureau of Fisheries, Washington, D. C., which has supplied the fish used in these experiments through their Forest Park Station, Saint Louis, Missouri. We are personally indebted to George A. Tamerassen, Director of the Forest Park Station, for his willing cooperation. Professor George W. Bartelmez of the Department of Anatomy of the University of Chicago has contributed invaluable advice concerning the anatomical relationships in the *Ameiurus* medulla.

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AVAILABILITY OF LACTIC ACID FOR BRAIN OXIDATIONS*

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THE FOOD requirements of the brain appear to be more limited than those of other organs. Whereas most parts of the body oxidize a mixture of both fat and carbohydrate, it has been repeatedly demonstrated that the R.Q. of both animal (1) and human (2) brain tissue is close to unity. There are three foodstuffs normally present in the body which yield an R.Q. of 1.0 on oxidation, namely, acetoacetic acid, glucose, and lactic acid. Goldfarb and Himwich (3) have shown that even with the high blood acetone levels of diabetic dogs the brain, in contrast to other organs, did not remove acetone substances from the blood. This indicates that acetoacetic acid, at least in the diabetic, does not serve as a substrate for brain oxidation.

All workers are agreed that brain oxidizes glucose, but the evidence for the oxidation of lactic acid is at present contradictory. *In vivo* studies reveal the removal of relatively small amounts of lactic acid from the blood traversing the brain whenever the level of blood lactic acid is raised (1, 4). However, under such conditions it is impossible to decide whether the lactic acid absorption is due merely to diffusion into the brain because of the higher concentration in the blood, or whether lactic acid is also actually metabolized by that organ. The second possibility is rendered doubtful by observations made on human subjects with basal values for lactic acid. Under these circumstances no absorption of lactic acid was revealed even during hypoglycemia when the brain is deprived of glucose (5). Mann and Magath (6) observed that lactic acid injected into hepatectomized dogs did not alleviate hypoglycemic convulsions, while glucose readily relieved the seizures. Studies of brain potentials in hypoglycemia have contributed valuable evidence on various aspects of brain oxidations. Hoagland, Rubin, and Cameron (7) showed that there are characteristic changes of the cerebral potentials of the cortex during insulin hypoglycemia. These changes are associated with a diminished oxygen uptake of the brain (8), occur despite the presence of a moderate lactic acidemia and are reversed by glucose administration. There were similar variations in brain potential in dogs during hypoglycemia produced by evisceration and hepatectomy (Maddock, *et al.*, 9). These authors found that the electroencephalograms returned to normal after the administration of glucose, mannose, and maltose; but were unchanged following the

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administration of fructose, galactose, sodium succinate, sodium fumarate, sodium glutamate, sodium pyruvate, sodium hexose diphosphate, glyceric aldehyde, or a mixture of glyceric aldehyde and sodium glutamate. These results are contrary to those obtained *in vitro* by Warburg, Posener, and Negelein (10) and others, who found that the oxygen uptake of excised brain tissue is accelerated when either lactic acid or glucose is supplied as substrate.

In attempting to ascertain the function of lactic acid, one is met by the difficulty that the liver converts this substance to glycogen (11) which may then be broken down to glucose and released to the blood stream in that form. It would seem that crucial experiments to determine the utilization of lactic acid by the brain *in vivo* should include at least four factors: (i) increase of the level of lactic acid in the blood, either induced endogenously in the organism, or produced by the administration of that substance; (ii) absence of other substrates, as in hypoglycemic coma; (iii) evidence of utilization of lactic acid during hypoglycemia as indicated by increased utilization of oxygen by the brain, and clinical revival of brain function with recovery from coma; and (iv) elimination of the possibility of conversion to glucose by removal of the liver, or estimation of this possible source of error by actual determination of the glucose and lactic acid uptake by the brain.

METHOD

The metabolism of the brain *in vivo* was studied in both man and animals. The cerebral metabolism of the human subjects was estimated from analyses of the arterial blood entering the brain and the venous blood leaving the brain through the interval jugular vein. The arterial blood was obtained from either the brachial or femoral artery. The venous sample was taken from the internal jugular vein using the technique described by Myerson

Table 1. Effect of 20 g. of sodium lactate intravenously on cerebral metabolism of schizophrenic patients in insulin coma.

Control before lactate

Exp. no.	Vol. per cent oxygen			Mg. per cent glucose		Mg. per cent lactate		Circ. time
	Art.	Ven.	Diff.	Art.	Ven.	Art.	Ven.	
1	18.5	13.6	4.9	43	52			12
2	18.6	16.8	1.8			26		8
3	18.7	17.9	0.8	47	45	38	32	14
4	15.8	13.9	1.9	40	42	40	35	11
5	17.3	16.1	1.2	35		29	17	9
6	18.7	16.4	2.3	39	36			11
7	21.5	18.3	3.2	56	42	25	36	8
8	20.7	17.0	3.7	55	48	32	19	9
9	20.2	14.5	5.7					13
10	18.5	16.4	2.1			45	34	10
11	20.2	19.9	0.3					9
12	22.6	17.2	5.4			37	36	10
13	18.8	18.1	0.7	41	26	18	4	16
14	19.9	16.0	3.9	35	25	28	10	8
			2.71	43	40	32	25	10.6

From 5 to 20 min after lactate

Exp no	Vol per cent oxygen			Mg per cent glucose		Mg per cent lactate		Circ time	Min after
	Art	Ven	Diff	Art	Ven	Art	Ven		
1	16 5	13 6	2 9	42	39			10	6
3	17 3	13 4	3 9	33	27			12	5
4	17 1	14 1	3 0	41	49	96	59	12	7
7	20 2	13 9	6 3	50	23	60	62	8	10
8	19 9	15 0	4 9	45	38	54	40	11	18
9	18 4	13 9	4 5					13	8
11	19 5	15 4	4 1					9	15
12	19 6	15 2	4 4			94	77	10	7
13	17 8	14 5	3 3	35	32	70	51	9	10
14	17 4	14 6	2 8	28	13	72	78		8
			4 01	39	32	75	61	10 4	

From 25 to 50 minutes after lactate

Exp no	Vol per cent oxygen			Mg per cent glucose		Mg per cent lactate		Circ time	Min after
	Art	Ven	Diff	Art	Ven	Art	Ven		
1	16 4	13 3	3 1	40	38			8	25
	17 3	11 4	5 9	40	48			9	45
2	19 1	14 9	4 2	58	55	42	44	8	25
	21 8	15 1	6 7	59	52	21	26	8	50
3	18 5	14 1	4 4	33	17	70	55	10	25
4	17 0	12 8	4 2	39	44	54	54	9	30
5	18 6	13 7	4 9	28		16	41	7	25
6	17 7	12 4	5 3	56	43			8	23
7	20 6	15 2	5 4	40	16	48	44	10	45
8	19 3	15 0	4 3	45		50		11	30
10	18 6	13 5	5 1			84		10	33
12	20 5	14 3	6 2			53		11	27
14	17 4	13 8	3 6	27	16	32	27		32
			4 87	42	37	47	42	9 1	

Halloran and Hirsch (12) The blood samples were analyzed for carbon dioxide, oxygen (13), lactic acid (14), and glucose (15) The velocity of the blood flow through the peripheral circulation was measured with the cyanide method of Robb and Weiss (16) The availability of the lactic acid was estimated in human subjects while the patients were in deep insulin coma, and again at various intervals after administration of 20 g of r-sodium lactate in 14 experiments, according to a method previously described (17)

In the animal experiments, dogs were hepatectomized under ether anesthesia, and were studied after they had completely recovered from the anesthetic The liver was extirpated by the one stage method of Markowitz, Yater, and Burrows (18) The intestines, spleen and pancreas were simultaneously removed In the various experiments glucose and lactic acid were injected intravenously Blood samples were analyzed for glucose (19), lactic acid, and oxygen

RESULTS

The experimental data on human subjects are presented in Table 1. The average oxygen uptake during coma, before the administration of lactate,

was 2.71 vol. per cent, the glucose uptake was 3 mg. per cent, and the lactate uptake was 7 mg. per cent. Observations made within the first 20 min. after intravenous injection of 20 g. of racemic sodium lactate revealed a moderate increase in the oxygen and glucose uptake, and a large increase in lactic acid uptake. Studies made from 25 to 50 min. after injection revealed a slightly larger oxygen uptake, and a return of the lactate uptake toward the earlier value despite the high blood lactic acid levels persisting at this time.

Table 2. Changes in glucose and lactic acid in hepatectomized dogs.

	Sugar		Lactic acid		
	Art.	Ven.	Art.	Ven.	
Dog No. 1					
45 min. postoperative	55	47	82	81	Convulsions 8 min. after 4 g. glucose recovery
2 hours postoperative		18		96	
2 hours 10 min. postoperative	138	141	110	104	
Dog No. 4					
55 min. postoperative	70	65	67		Convulsions
2 hours 5 min. postoperative	34	26	98	101	
Dog No. 6					
1 hour 20 min. postoperative	25	19	78		Convulsions 8 min. after 6 g. glucose recovery 53 min. after 8 g. sodium lactate
1 hour 40 min. postoperative	250	195	135		
3 hours 12 min. postoperative	27	17	162	163	

The clinical condition of only two patients showed any change. In experiment 2 there was a definite diminution of the depth of coma, while the patient in experiment 7 aroused sufficiently to answer questions. The latter was fairly well oriented, but after 20 min. again lapsed into coma. This patient habitually roused from coma after any manipulations. In both these cases the arteriovenous oxygen differences approached the normal during the period of arousal. The remaining patients showed no clinical change. The cyanide circulation time showed no significant change.*

The results of the animal experiments are presented in Table 2. Seven dogs were studied. Dogs No. 1, 2, and 3 had high levels of lactic acid as a result of the ether anesthesia, which further increased after the hypoglycemic convulsions. Despite the lactic acidemia, convulsions occurred as hypoglycemia developed. Furthermore, the convulsions were immediately relieved by the injection of glucose. When glucose injections were withheld from dogs No.

* Similar experiments subsequently performed, with brain blood flow simultaneously recorded with a modification of the Gibbs thermostomat revealed no significant changes in brain blood flow in the course of the experiments (see HIMWICH, H. E., BOWMAN, K. M., DALY, C., FAZEKAS, J. F., WORTIS, J., and GOLDFARB, W., Changes in cerebral blood flow and arteriovenous oxygen difference during insulin hypoglycemia. *Proc. Soc. exp. Biol.* N. Y., 1940, 45: 468-469.)

4 and 5, the high level of lactic acid did not save the animals. Dog 6, which recovered from hypoglycemic convulsions after intravenous glucose administration, succumbed later to hypoglycemia despite the intravenous injections of sodium lactate. A similar observation was made with dog 7.

DISCUSSION

Estimations of brain metabolism by oxygen, glucose and lactic acid differences in the arterial and venous blood are subject to two sources of error, (i) diffusion of substances between tissue and blood due merely to changes in concentration, and (ii) changes in blood flow through the brain. The first of these factors could not be measured, but since the determinations were made at varying intervals after the administration of lactate, it seems probable that the factor of diffusion would have resulted in variable results in both directions.

The second possible source of error involved in this method of studying brain metabolism has been recently emphasized by Abramson *et al.* (20), who believed that the diminished oxygen uptake observed in insulin shock may be attributable to changes in blood flow. These authors noted an increased blood flow in the extremities of 6 of 11 patients receiving insulin therapy for schizophrenia. Despite the fact that brain blood flow was not recorded, they concluded that their evidence indicated that there might well be an increased blood flow through the brain which would account for the fall in the arterio-venous oxygen differences. Inasmuch as our determinations as well as those of Himwich *et al.* (5), were made during actual coma, approximately 4 hours after insulin injection, and the observations of Abramson *et al.*, were made between 60 and 115 min. after injection, our data are not comparable. The latter authors state: "For the most, the various symptoms associated with hypoglycemia appeared only after an increase in peripheral blood flow was noted." Moreover, to explain a fall of the average arterio-venous difference from 7 to 2 vol. per cent, an increase of the rate of blood flow of 350 per cent would be required. It is doubtful whether so great a change could occur in the brain blood flow under these conditions. Direct measurements of cerebral blood flow during hypoglycemia reveal no such changes. In man a gradual slight decrease of brain blood flow during hypoglycemia has been reported by Loman and Myerson (21). Such a result is in agreement with the fact that there is a gradual fall in blood pressure as the hypoglycemia develops. Studies by Leibel and Hall (22) on rabbits during hypoglycemia reveal no significant changes in blood flow unless convulsions ensue. Because of these direct observations of a decreased or unchanged brain blood flow, and because of the fact that the patients become comatose, we believe that the smaller arterio-venous differences indicate a diminished brain metabolism.

In the present experiments evidence has been presented indicating that the brain tissue *in vivo* cannot oxidize lactic acid sufficiently to sustain cerebral function. In the hepatectomized animals the administration of lactate

did not serve to alleviate the hypoglycemic convulsions at once. These results are in agreement with those of Mann and Magath (4). Similarly, the comatose state associated with insulin hypoglycemia in man could not be relieved by the administration of lactate. The oxygen uptake of the brain in man, which is diminished in hypoglycemic coma, was only slightly increased despite the fact that large amounts of lactate were removed from the blood. It is well known that the liver may convert lactate to glycogen (6) which, after conversion to glucose, may serve to elevate the blood sugar. This mechanism for the glucose lactic acid cycle functions with high levels of blood lactate under postabsorptive conditions (24) but the rate of liberation of glucose by the liver is not sufficiently great to cope with the cerebral requirement for glucose. On the other hand, after hypoglycemia of 5 hours duration, Himwich *et al.* (23) found that the injection of 4 g. of glucose diminished the depth of coma and 8 g. of glucose caused the reappearance of both consciousness and the alpha waves of the electroencephalogram. In a separate group treated at Bellevue Hospital, 4 g. of glucose invariably roused the patient after the onset of coma, and restored the oxygen uptake from 2.9 to 5.5 vol. per cent approximating the precomatose levels (26). This is contrary to the evidence derived from the studies of oxidations of excised brain tissues. The possibility remains, however, that the oxidations *in vitro* occur at a diminished rate (25) and may be maintained by lactate oxidations, while the oxidation of lactate *in vivo* is too slow to support cerebral metabolism during hypoglycemia.

SUMMARY AND CONCLUSIONS

Twenty grams of r-sodium lactate were injected into patients during therapeutic hypoglycemic insulin coma. The injected lactate did not support brain oxidations sufficiently to rouse the patients, and the oxygen uptake of the brain showed only a moderate increase.

Seven dogs were hepatectomized under ether anesthesia, and were studied after they recovered from the anesthesia. Lactic acid, whether formed by the animals or administered intravenously, did not serve to prevent hypoglycemic convulsions or to alleviate them once they were initiated. This failure contrasts strikingly with the effects of glucose and indicates that the rate of oxidation of lactic acid in the brain is not sufficient to adequately support cerebral functions.

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CORTICAL EXTINCTION IN CONVULSIONS*

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INTRODUCTION

THE PURPOSE of the present communication is to demonstrate that (i) following an epileptiform seizure, whether induced by electrical stimulation or occurring "spontaneously," the electrogram of the human cerebral cortex discloses for a period a condition predominantly isoelectric; (ii) during this period of isoelectricity a stimulus of supraliminal value applied to the cortex is ineffective for the production of changes (a) in somatic motor activity and (b) in the cortical electric potentials; (iii) the slow restoration of cortical excitability (as measured by the motor response to stimulation) is signalled by a displacement of the isoelectric state; and (iv) an exalted state of cortical excitability probably then supervenes. These phenomena have not been recorded heretofore. That the qualitative and quantitative data derived from the present inquiry closely resemble or are identical with those of "extinction," as demonstrated in the less vigorous physiological activity of the sub-human nervous system, will become evident as the experimental findings are described.

The phenomenon known as *extinction* was discovered in 1934 by Dusser de Barenne and McCulloch (6). Its demonstration has proven of value to neurophysiology, contributing notably to progress in the study of the processes of neural decrement, inhibition, fatigue and oscillation just as the demonstration of facilitation by Bubnoff and Heidenhain (3) in 1881 contributed to an understanding of the processes of excitation. As defined, extinction is a diminution or absence of response observable (within an interval longer than that required for facilitation) upon repetition of stimulation of a "motor" focus of the central nervous system (9). Its influence may be observed as early as from several seconds to one-half a minute after the application of the stimulus responsible for its initiation and it may continue to exert its effect in some cases for as long as half an hour. Thus, a stimulus applied to a physiologically circumscribed region, e.g., the hand moiety of the premotor cortex in Brodmann's area 6a α , so alters the functional condition of the central nervous system that when later a test stimulus is reapplied to that region, the measured response is either diminished or completely extinguished. A similar diminution or absence of response is observable when the test stimulus is applied to the immediately adjacent cortex and to cortical regions spatially more remote but functionally related to the hand region (e.g., in the corresponding regions of Areas 4, 3, 1, 2 and 5). The diminution in response referred to may be manifest as a rise in threshold of excitability, an increase in the latent period between the application of

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stimulus and the first appearance of a measurable response, a decrease in amplitude or response, or any combination of these * Extinction obtains as well with stimuli of subliminal value as with those of supraliminal value and in this respect it is comparable to the phenomena of summation and occlusion as they relate to facilitation The more recent experiments of Dusser de Barenne, McCulloch and their associates (7, 8, 9) clearly demonstrate that the following factors attend extinction (i) hypoactivity resulting in less summation, (ii) positive voltage drift associated with increase of threshold in neurones previously involved, and (iii) acid shift (decrease of pH), increasing the threshold of all neural structures in the region involved

The observations presented here relating to seizure potentials of the exposed human brain were begun in September, 1938 They were originally undertaken with the intention of seeking a method which might supplement and render more precise the data obtainable from other sources bearing on the localization of epileptogenous foci Prior to this time, it had been the frequent experience of the writer at operation that, following a convulsive seizure elicited by an electrical stimulus of relatively low strength, the cortex remains for a variable period of time quite unresponsive to the reapplication of a stimulus of the same or higher value An attempt was therefore made in the present inquiry to obtain quantitative information relative to the two major types of change which are produced by antecedent stimulation, namely, alteration in threshold, as revealed by the determination of the minimal stimulation which produces an electrical response at the region under study, and alteration in activity as revealed by the electrical potential records of the cortex

The number of studies in which the bioelectric potentials of the exposed human brain have been recorded for any purpose is unusually small (13, 23, 24, 26, 27) Foerster and Altenburger (13) were the first and seemingly the only investigators to record from the exposed human cortex electrical potential changes incidental to a seizure

The literature discloses a few observations which bear more or less directly on the problem on hand Thus, the experiments of Fischer (12), and of Goodwin, Kerr and Lawson (21) on the exposed animal brain and the observations of Berger (2), Gibbs, Davis and Lennox (16, 17, 20) and Davis and Sulzbach (5) on man with intact cranium demonstrate that following both drug-induced and spontaneous seizures the cerebral potentials are for a period "flat" This finding came to be interpreted as representing a decrease in bioelectric activity, running more or less parallel with the subsidence of muscular activity and prevailing for a period corresponding roughly to the duration of post convulsive stupor In addition to these findings, the observations of Fender (11) on dogs and of Clark and Ward (4) on cats coincide generally with the observations of the present writer to the effect

* It is of course essential that the reader bear in mind the physiological characteristics which differentiate *refractory period*, *suppression* and *inhibition* (in Sherrington's sense of the term) from *extinction*

that cortical stimulation at intervals after an induced seizure yields "less complete seizures" even though a stronger stimulus than that which first proved effective was employed. Quantitative data were not recorded in these animal experiments.

MATERIALS AND METHODS

Ten human subjects constitute the basis of the present study. Seven were males and three females. The age of the youngest patient (J.C.) was 12½ and of the eldest (F.O.) 32 years. All experiments were performed during the course of operative procedures for paroxysmal convulsive disorders at the Kings County Hospital.

On the basis of preliminary studies consisting of the anamnesis, the characteristic sequence of events of the seizure pattern, the neurological findings, roentgenography, pneumoencephalography and electroencephalography, the site of the epileptogenic focus was postulated in each case. Sedatives were avoided during the two days before operation. The scalp was infiltrated with procain hydrochloride, an osteoplastic bone flap was turned down and the dura reflected. A careful sketch of the disposition of the sulci, gyri, cortical vessels and sites of pathological alteration (if any) was made and some seven to ten "zones" (depending upon the magnitude of the area of the exposed cortex) were arbitrarily defined on the cortical surface as points of reference. These zones were indicated on the sketch.

A control electrographic record was now made for each separate zone, the electrical potentials being picked up by means of electrodes and a holder especially designed for this purpose. Under certain conditions, the familiar co-axial type of electrode of Adrian and Bronk (1) was used. The brain potentials were led through a single-channel electroencephalograph and, after amplification, were recorded simultaneously by two means, viz., a cathode ray oscillograph, the standing waves of the sweep circuit of which could be observed by the surgeon directly and the oscillations of which could be recorded cinematographically; and a push-pull type of ink-writing oscillograph, the oscillations of which could be recorded directly on a moving paper tape. The basic design of this instrument has been described by Garceau and Davis (14, 15).

After obtaining control records of the various zones in the manner above described, an attempt was made to identify more precisely the epileptogenic focus ("firing point") presumed to lie within the operative field. For this purpose stimulation of the cortical surface was carried out beginning with values well below threshold and working up by successive increments until the limen for eliciting a motor response from the excitable motor cortex of the face and/or hand area was ascertained. The exposed cortex was then systematically explored with this strength of stimulus, the intention being to discover a region from which a seizure could be produced that simulated in its subjective and objective features the so-called spontaneous seizures of the patient. If this stimulus value failed to produce a response it was gradually increased until the desired result was obtained. The instrument employed for this purpose was a 60 c./sec. bipolar stimulator. In the first 4 experiments an A.C. sinusoidal wave form having a period of 16.6 msec. was used. In the other 6 experiments an interrupted D.C. parabolic type of wave having a period of 8.3 msec. was employed through the intermediation of a copper rectifier. The bipolar stimulating tips consisted of silver balls of 1.5 mm. diameter separated 2-3 mm. from each other. The stimulating tips were applied for approximately 3 sec. An interval of 10-14 sec. was allowed to elapse between successive applications of the stimulating electrodes to the cortical surface, the purpose of this delay being to permit the effects of induction and facilitation to pass off.

A trained observer was posted under the sterile drapes for the purpose of reporting to the surgeon the patient's responses to stimulation. All observations were recorded stenographically and were correlated with the electrographic tracings, particular attention being given to temporal factors. After each application of a stimulus, the pick-up electrodes were applied to the corresponding cortical zone. Whenever a seizure was initiated, the electrographic record was continued until an effectually normal tracing of cortical potentials reappeared. Meanwhile, the effect of reapplying stimuli of varying supraliminal as well as liminal strengths was noted at regular intervals.

RESULTS

The essential conformity of the descriptive data derived from the 10 experiments permits a treatment of the series as a whole. In the interest of space conservation, therefore, the findings are summarized in Table 1. Only those protocols (or portions thereof) will be presented which appear to be

Table 1 Summary of the data derived from the 10 experiments of the present series bearing on the phenomena of cortical extinction and isoelectric state (Period of extinction includes both absolute and relative phases, Ineffective refers to failure to produce a major convulsion, Undet = undetermined)

Exp t No	Patient	Value of stimulus evoking convulsion	Duration of period of extinction	Highest value of stimulus ineffective during extinction	Exalted state following extinction
I	J C	0.65 mA 4.5 V	19 min	2.25 mA 9.0 V	Yes
II	W G	1.5 mA 6.5 V	11 min	2.25 mA 9.0 V	Undet
III	H H	1.0 mA 5.5 V	21 min	2.10 mA 8.5 V	Undet
IV	T C	0.45 mA 4.0 V	25 min	2.5 mA 10.0 V	Yes
V	M W	0.20 mA 2.5 V	14 min	2.1 mA 8.5 V	No
VI	P B	0.10 mA 1.0 V	21 min	2.35 mA 9.5 V	Yes
VII	M R	1.30 mA 6.0 V	9 min	2.35 mA 9.5 V	Undet
VIII	A P	0.65 mA 4.5 V	17 min	2.25 mA 9.0 V	No
IX	F O	2.25 mA 9.0 V	19 min	3.25 mA 13.0 V	Yes
X	J S	1.7 mA 7.0 V	22 min	2.35 mA 9.5 V	Undet

of direct value in supplementing the table and in illustrating the major points under consideration in this communication.

Experiment I Patient J C, a schoolboy aged 12½ years, was admitted for the second time to the Kings County Hospital on September 8, 1938. The neurological findings, the sequence of events of a typical seizure and the electroencephalographic tracings appeared to justify the supposition that an epileptogenic firing point might be discoverable high on the convexity of the right hemisphere in either Area 6 or Areas 3, 1, 2, 5 or 7. Accordingly on September 26, 1938 a bone flap was reflected. The exposed cortex, substantially normal to gross inspection, presented an appearance as illustrated in Fig. 1. A small focal area of

cortical atrophy with a correspondingly deep subarachnoid lake was noted near the junction of the precentral sulcus and the posterior limit of the middle frontal convolution. Cortical electrograms were recorded from each of the 9 zones (Fig. 1) after which stimulation was begun. No responses were obtained until a stimulus of 0.12 mA. and 1.5 V. was

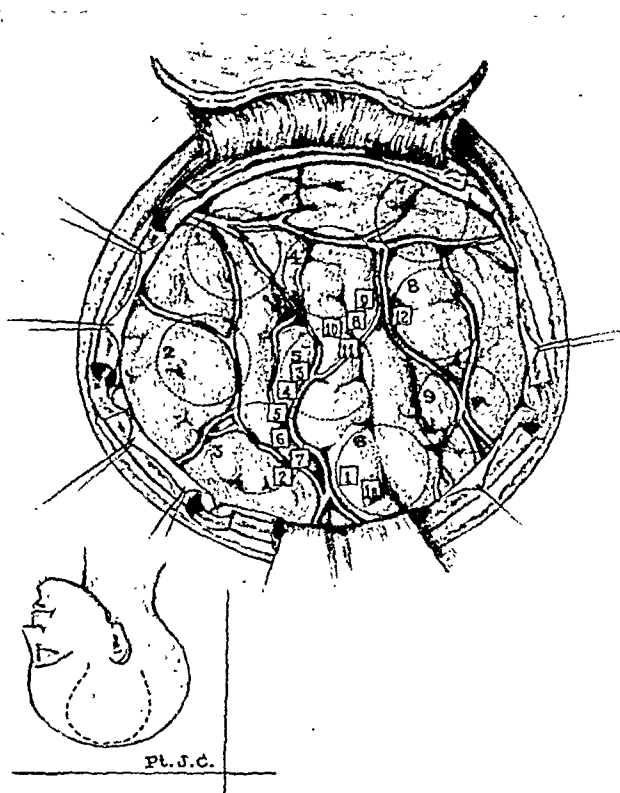


FIG. 1. The operative field of patient J. C. The numbers within the dotted circles indicate the 9 arbitrary zones from which control electrograms were recorded. The arrow points to a small region of cortical atrophy. The numbers within the squares indicate the points from which responses described in the protocol were obtained.

employed. This stimulus was everywhere ineffective except at the point marked 1. From here, movements of the left upper extremity closely simulating those characteristic of the earliest motor events of the patient's spontaneous seizures and lasting 12 sec. were elicited. This stimulus and stimuli at graded increments ranging up to 1.5 mA. and 6.5 V. were wholly ineffective during the next 17 min. when applied at intervals of 14 sec. to all parts of the exposed cortex. At the end of this time, the originally effective stimulus again proved capable at point 1 of exciting a response, this time of 5 sec. duration, in the left upper extremity. The pattern of this response was as previously observed. The stimulus was now set at 0.30 mA. and 3.5 V. The cortex in and adjacent to point 1 was purposely avoided until the other parts of the cortex should be explored. At the point marked 2 the patient reported experiencing "a feeling as if I'm going to go." There was a slight and brief movement of flexion of all fingers of the left hand but the episode was arrested here. This same value of stimulus proved effective for the elicitation of slow tonic flexion movements of the

individual digits when it was applied to the points marked 3, 4, 5, 6 and 7 in Fig 1. Re-stimulation of each of these points at an interval of 15 min following the first reaction elicited a response of slightly less magnitude. The same strength of stimulus was now applied to the point marked 8 where it evoked a quivering of the lower lip of 3-4 sec duration. As soon as the quivering ceased, the point was restimulated and a like response was again observed. This time, however, it lasted for 7 sec (facilitation). Reapplication of the stimulus to region 8 and to the surrounding regions (9, 10, 11, 12) was without effect 5 min later.

Pt J C, Age 12 yrs

50 Microvolts (appr)

Time in Seconds

Control Record, Zone 6

Zone 6, Point 1a St

TONIC
L HAND

GENERALIZING

ENDED
52 sec

Tw's
rt f
up ext

Zone 6 Vomiting & Retching

artf

artf

artf

artf

artf

FIG 2 Cortical electrogram from zone 6. Stimulation at point 1a with 0.65 mA and 4.5 V elicited a convulsion starting with a tonic contraction of the left hand and marching into a generalized seizure. The duration of the electrically recorded seizure was 52 sec. During the ensuing isoelectric period twitches of the right face and upper extremity were still observable (tw's rt f & up ext). The tracings during retching and vomiting and the succeeding isoelectric state are shown (artf = artifacts due to gross head movements).

The stimulus values were now advanced to 0.65 mA and 4.5 V and the cortex was again explored, avoiding at the beginning the regions from which the above described responses were obtained. When the stimulus was applied to point 1a, a major convulsion supervened the pattern of which closely simulated the spontaneous attacks of the patient. An electrographic tracing of zone 6 was made beginning at the moment when the tonic extension of the left hand was reported. The electrically recorded attack lasted for 52 sec. In spite of the fact that following this attack the tracings from zone 6 appeared isoelectric, a few irregular twitches of the right face and right upper extremity were observable during the next 8-9 sec. The isoelectric state persisted for some 12-13 sec and then the patient began to retch and vomit. The record obtained at this time was irregularly interlarded by readily recognizable artifacts, obviously due to coarse movements of the head. As soon as the retching subsided, however, the condition of essential isoelectricity again prevailed (Fig 2).

During the immediately succeeding 15 min that strength of stimulus which evoked the described response was again applied to point 1a. No clonic features were noted in this action.

The patient was now restless and occasionally moaned and mumbled. The stimulus was reduced to its previously liminal value of 0.65 mA. and 4.5 V. It now proved ineffective when applied at intervals of 1 min. to the cortex in and around point 1 until the seventh minute following the termination of the convulsion. The cortical tracings at this time were still relatively isoelectric, but a response was nevertheless obtained, consisting of a progressive tonic spread of the fingers of the left hand, an extension of the hand and an abduction movement of the arm. This tonic action lasted for 1-2 sec. and was followed by a few clonic jerks, the entire episode lasting approximately 5 sec. The electrogram disclosed a corresponding interruption of the isoelectric tracing (Fig. 3). Evidence of increasing cortical

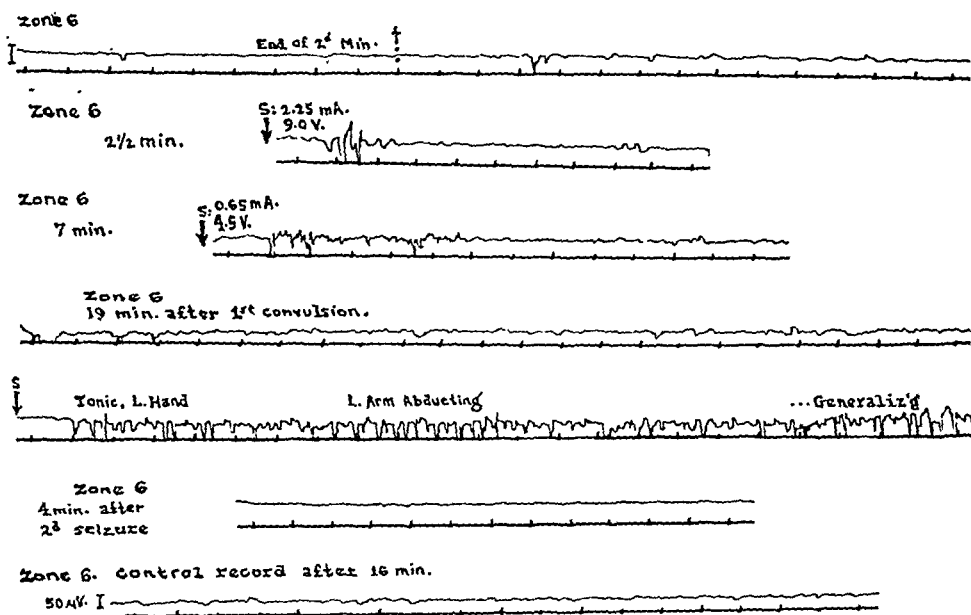


FIG. 3. Cortical electrograms from zone 6. The prevailing isoelectric state following the convulsion of Fig. 2 beings to break (right half of top line). Following the seizure by 2.5 min. application of a supraliminal stimulus of 2.25 mA. and 9.0 V. to point 1 elicited a brief and weak response of the left upper extremity. At the end of 7 min. the liminal value of stimulus, 0.65 mA. and 4.5 V., excited from point 1 an "aborted" attack lasting 5 sec. At the 19th minute, autonomous potentials closely resembling those of the control period entirely replaced the isoelectric tracing. At this time, the liminal value of stimulus elicited a major spell of greater severity than the first, lasting 88 sec. Only the earliest part of the tracing is shown here. A sample of the isoelectric tracing taken 4 min. after the second convulsion follows. The lowermost strip demonstrates returning potentials 16 min. after the attack.

excitability was obtained from this time on and by the fifteenth minute more nearly autonomous tracings began to appear. When, at the nineteenth minute, the latter appeared to be well established, the originally liminal stimulus (i.e., 0.65 mA. and 4.5 V.) was applied to point 1 and this time a second major convulsion was evoked which, in its pattern, closely simulated that first elicited. From the clinical standpoint this attack was more severe than the first, as judged by its greater duration (88 sec.) and by the vigor and amplitude of the convulsive movements. After the cessation of this seizure, an isoelectric state again prevailed not only in zone 6 but in the other zones of the exposed vortex (Fig. 3). Sixteen minutes later, the electrogram revealed a return of more nearly normal autonomous potentials.

Experiment IV Patient T C, a plumber aged 28 years, was admitted for the second time to the Kings County Hospital on November 9, 1938 with a history of convulsions refractory to medical treatment for 11 months. The attacks invariably began with persistent twitching of the left eyelids and were either aborted here or progressed thence in a physiologically orderly march into a generalized convulsion. Electroencephalography disclosed a dysrhythmia of the right frontal and temporal regions the pattern of which did

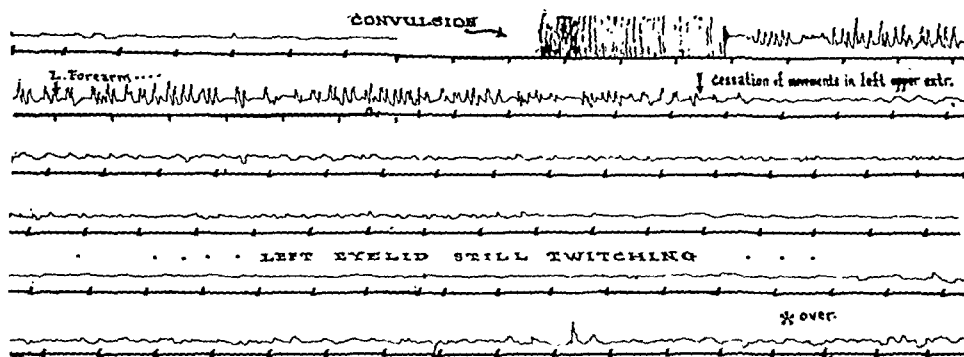


FIG 4 The operative field of patient T C. The numbers within the dotted circles indicate the 8 arbitrary zones from which control electrograms were recorded. The 2 arrows point to small opaque cortico-arachnoid cicatrices. The numbers within the squares indicate the points from which responses described in the protocol were obtained.

not conform closely to any well defined pattern (17, 18, 19, 20, 22, 25). The sum total of clinical evidence . . . in Area 8b of the right frontal lobe. On November 22, 19 . . . effected. The cortex appeared essentially normal except for a dense, opaque sclerotic patch located at the junction of the precentral and inferior frontal gyri. This seemed to draw a number of the local vessels into a pit. A similar patch was noted 1.5-2 cm. superior to the first (Fig 4). Control electrograms were made of the eight zones indicated on the sketch and stimulation was begun with 0.20 mA and 2.5 V. No responses were elicited until these values were increased to 1.0 mA and 5.5 V. At point 1 this strength of stimulus elicited a twitching of the upper and lower lips on the left side which persisted for 55 sec. The left eyeball went through irregular rhythmic jerks at this time, seemingly independent of its fellow. Five minutes later the same stimulus, when applied to point 2, elicited twitching movements of the left forehead, cheek and eyelids and a clonus of the platysma myoides. This response lasted but 25 sec. At the onset

the patient reported a sensation "as if" he were turning. The stimulus was now reduced to 0.45 mA. and 4.0 V. At point 3, fluttering movements of the left eyelids were produced, succeeded after 9 sec. by a few clonic jerks of the left forearm of 11 sec. duration (Fig. 5). Stimulation of the same point at the end of 2 min. resulted in only a few fluttering motions of the left eyelids, the episode lasting but 8 sec. Ten minutes later the same stimulus when applied to point 4 elicited clonic twitches which appeared almost simultaneously along the left upper extremity, from the fingers to the shoulder. After another 10 min., stimulation at point 5 evoked a seizure which closely simulated the patient's usual attacks. The episode lasted 2.5 min. The electrogram disclosed oscillations of high frequency and large amplitude, gradually becoming isoelectric (Fig. 6). Systematic stimulation of the exposed brain during the ensuing 22 min. was entirely ineffective with the same stimulus and with stimuli ranging up to 2.5 mA. and 10 V. The electrogram now began to show an irregular return of

ZONE 5,
POINT 3 St.



Pt. T. O., 11-22-'38.

FIG. 5. Cortical electrogram from zone 5. Stimulation at point 3 with 0.45 mA. and 4.0 V. evoked fluttering movements of left eyelids followed after 9 sec. by irregular clonic jerks of the left forearm (L. Forearm . . .). Note eyelids still twitching during a prevailing isoelectric state in this zone. Asterisk denotes cessation of all observable twitching.

autonomous oscillations. Therefore the stimulus values were reduced again to the previously determined liminal values of 0.45 mA. and 4.0 V. At the twenty-fifth minute, this stimulus, elsewhere ineffective, was in the process of approaching point 5. When it was applied to point 6 a severe convulsion, far more violent in the vigor and amplitude of its clinical features than the previous seizure, was evoked. Again, a period characterized by an isoelectric state supervened (Fig. 7). This state was altered at times by slow voltage drifts and was replaced after 19 min. by more nearly normal autonomous waves. At the twentieth minute following this attack, while observations of the post-convulsive period were still being made, a "spontaneous" seizure appeared, lasting 65 sec. This was of the same pattern as noted in the two previous attacks, but clinically it appeared to be the most severe of all. The cortical electrogram corresponding to the succeeding post-convulsive period disclosed an isoelectric state, demonstrating that this condition of the cortex follows "spontaneous" as well as induced seizures (Fig. 7).

Experiment V. Patient M. W., a laborer aged 31 years, was admitted to the Kings County Hospital on March 29, 1939 complaining of convulsive seizures of 21 years' duration. The onset of the attacks was characterized by electric-like shocks in the right hand and forearm, numbness and tingling of all fingers of this limb, and severe clonus of the limb. The arm then twisted behind the back, the head rotated to the right and the right eyelids twitched. The attack was either aborted here, in which case consciousness was not lost, or it became rapidly generalized, in which case unconsciousness supervened. Further details

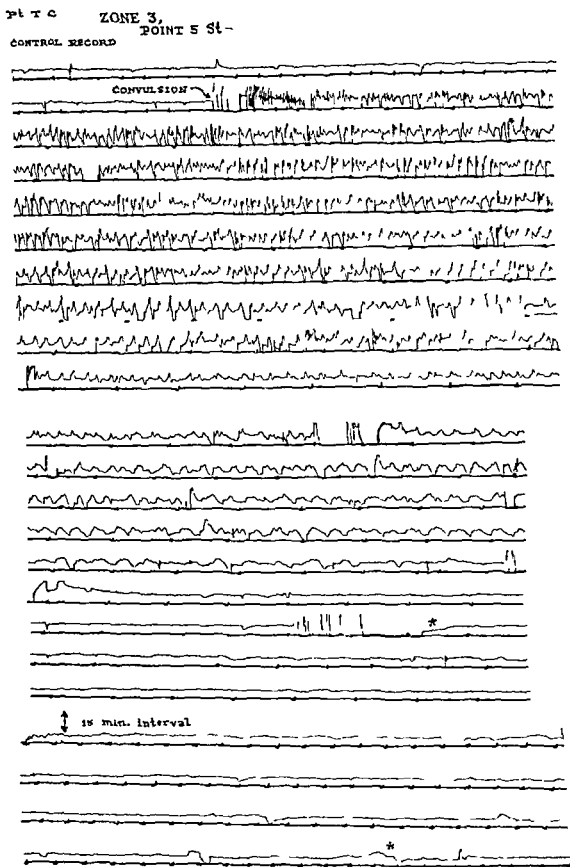


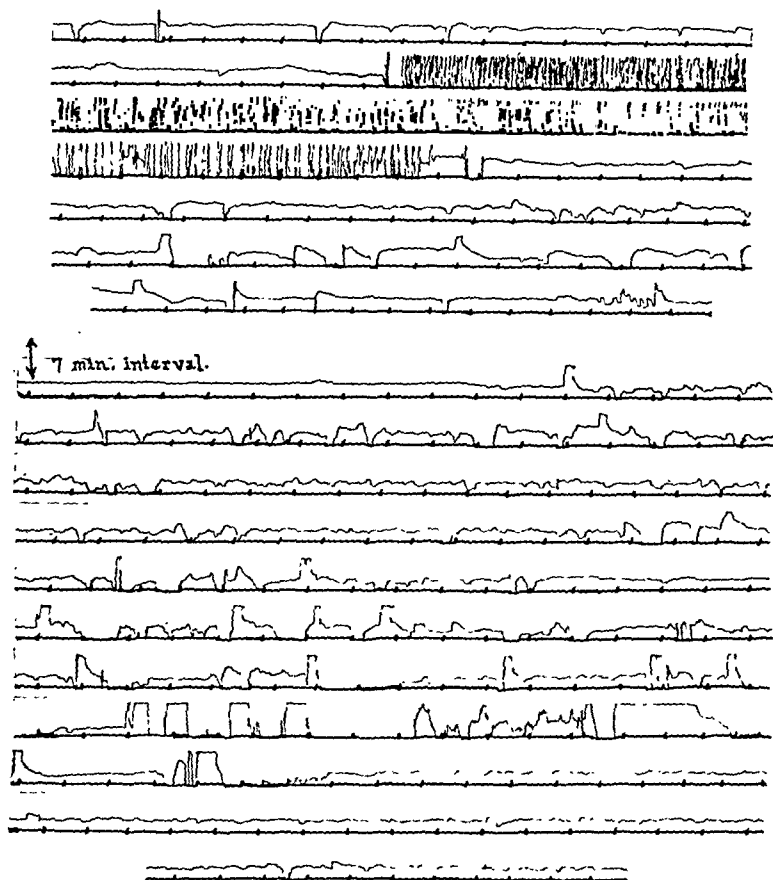
FIG 6 Cortical electrogram from zone 3, 22 min after response recorded in Fig 5. Stimulation at point 5 with 0.45 mA and 4.0 V elicited a seizure simulating the patient's usual attack. Liminal and supraliminal stimuli were ineffective during the succeeding 22 min.

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it is desired to illu

reflected. The operative region presented a dense broad cicatricial sheet, the sequel of an old cerebral contusion. The anterior edge of the scar proved to be the region from which,

joint
was

Et. T. C. ZONE 5, [induced convulsion]
POINT 6 St.



ZONE 5 [“spontaneous” seizure]

Calibration 50 μ v.
gain set at 50

39 min. since previous seizure

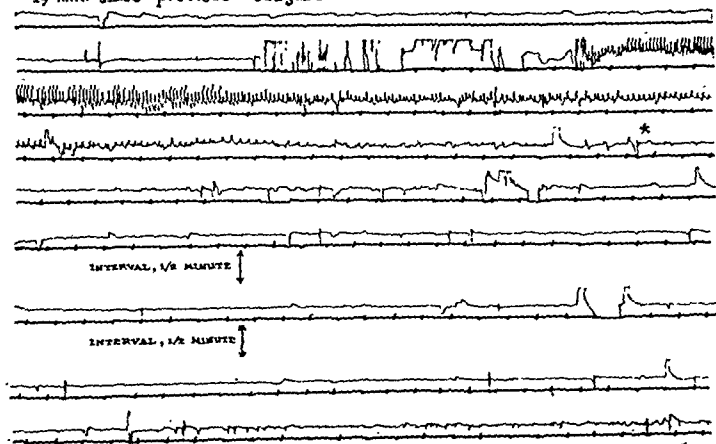


FIG. 7. See next page for legend.

FIG 7 Cortical electrogram from zone 5, 25 min after the attack recorded in Fig 6. Stimulation at point 6 with 0.45 mA and 4.0 V elicited a major seizure of greater severity than that previously evoked. Twenty minutes later a spontaneous seizure supervened, the most severe of all. Note prevailing isoelectric state following the latter attack.

with a stimulus of low strength (0.20 mA and 2.5 V) a convulsion closely simulating the patient's spontaneous attacks was elicitable. As indicated on the record, during the isoelectric period which followed this convulsion twitchings of the left side of the face were still observable (Fig 8). After the complete subsidence of the convulsion and throughout the succeeding 14 min stimuli of values ranging as high as 2.1 mA and 8.5 V proved made-

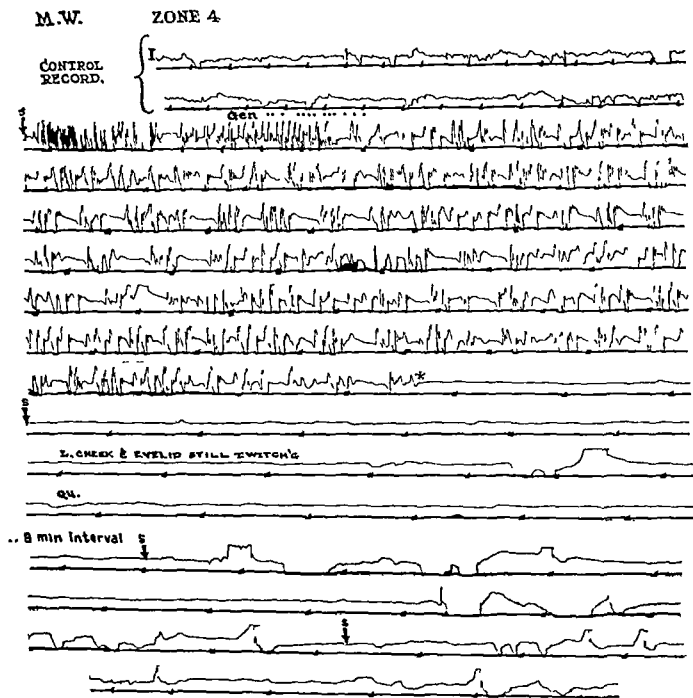


FIG 8 Cortical electrogram from patient M.W., approximately in Brodmann's area 44. Convulsion elicited by stimulation of point on anterior edge of cicatrix, using 0.20 mA and 2.5 V. Note that during the isoelectric state, twitches of left cheek and eyelids were still observable. I = 50 μ V. deflection, S = application of stimulus, Gen . . = convulsion becoming generalized, asterisk denotes end of generalized convulsion, Qu = entirely quiet.

quate to excite an evident motor or sensory response. The point to which particular attention is drawn by this experiment (as also by experiments I and II) is that motor responses were still observable at a time when the electrogram of the zone about the stimulated point appeared isoelectric. It seems reasonable to suppose that an electrogram taken from the homologous zone in the opposite hemisphere at this time would have disclosed a few waves of large voltage and rapid rate. Under the conditions of experimentation, however, the recording of such potential changes was not feasible.

DISCUSSION

The protocols are consistent from experiment to experiment and they appear to demonstrate the following points:

1. The strength of stimulus required to evoke a major convulsive seizure by stimulation of a "firing point" varies from case to case within rather wide limits (from less than 0.10 mA. and 1.0 V. to 1.2 mA. and 9.0 V.). This variation is such that in one patient a convulsion may be discharged by a stimulus the value of which is insufficient to excite even a simple response when applied to the motor cortex of another patient.

2. The strength of stimulus required to evoke a major convulsive seizure by stimulation of a "firing point" can be shown to vary from attack to attack in any given patient during the course of an operation. This variation is at least in large part if not entirely ascribable to the antecedent activity of the cortex. (a) The application of the stimulating electrodes carrying liminal and supraliminal values proved ineffective to excite a convulsion for an average of 17.8 min. after the offset of a previously elicited attack. (b) During the advancing course of this period, motor responses of a delimited order, usually characterized by the earliest events of the spontaneous seizure pattern and resembling the *aurae* and aborted seizures of the patient, can be elicited provided a stimulus of sufficiently high supraliminal value is employed. As the period progresses, a supraliminal stimulus of a given value elicits more and more vigorous responses or, expressed in another way, the supraliminal value required to evoke a response of given magnitude is in inverse proportion to the time. (c) Following the expiration of this period, stimuli of original liminal value are seemingly capable of eliciting a convulsive seizure of more vigorous character than that first evoked. This was apparent in four of the six experiments in which its demonstration was specifically sought. The two negative results indicate the necessity for reserving final conviction in this regard until further experimental data are at hand. It is conceivable, however, that in these two experiments the stimulus which proved capable of eliciting a second major convulsion was applied to the neighborhood of the firing point at a time somewhat too late to find the cortex still in an exalted state of excitability. In the other four cases attempts to demonstrate the phenomenon had to be abandoned for one reason or another dictated by the circumstances at operation.

3. The period during which the convulsive responses are either wholly incapable of elicitation or appreciably dampened is characterized by a relatively isoelectric state as revealed by the cortical electrograms. As the period progresses, the electrographic tracings indicate an irregular resumption of

cortical potentials which come more and more to resemble those observed during the control period. It appears that the electrical excitability of the cortex is recovered *pari-passu* with the subsidence of the isoelectric period until it at last becomes such that a second convulsion is elicitable. In this series the periods of absent or diminished response ranged between 9 and 25 min. and they were observable following both spontaneous and induced seizures. They could not be said to correspond precisely to the period of depressed psychological responsiveness (clinically, "unconsciousness," "post-convulsive stupor," etc.) inasmuch as fairly complex verbal responses were executed in some instances at the time when a relatively isoelectric state prevailed.

The evidence thus far arrived at appears to indicate that the phenomena underlying and following upon the discharge of a convulsion are qualitatively identical with those underlying other more typical physiological excitations. It therefore seems unnecessary to invoke any new principle in attempts to account for the spread of excitation in seizures, such as an electrical wave front advancing across the cortical surface without reference to neuronal interconnections, a vasospastic process, a hypothetical chemo-excitant, etc. On this point the writer concurs with Erickson (10) who adduced strong experimental evidence to the effect that the spread of cortical excitation in convulsions is mediated strictly by neurones and their processes.

Whether a positive voltage drift, a decrease in pH and an accumulation of lactic acid in the cortex are regular concomitants of the post-convulsive isoelectric state in the human will have to await demonstration until methods recently evolved in the animal laboratory can be adapted to the operating room. Nevertheless, the phenomena associated with the period of increased threshold observed in the present experiments resemble so closely those of extinction that eventual proof of their identity appears to be reasonably certain.

4. The exalted state of cortical excitability which seemingly follows the period of extinction in certain experiments is of additional interest. It suggests a partial account for the clinically described *status epilepticus*, viz., after a convulsion is spent, there supervenes a period during which the cortex is unresponsive to the substrate of physiological stimuli (e.g., hypoglycemia, alkalosis, anoxia, positive water balance, endocrine factors, etc., etc.) which tend to set off a new spontaneous attack; as the state of cortical excitability passes from one of absolute extinction to one of relative or partial extinction a second attack (of weaker character) may be discharged, provided only that the stimuli are still "set" at a sufficiently high level; if they are not so set they remain incapable of discharging a new seizure until the exalted state of cortical excitability following the period of extinction is reached. The process is then repeated. According to such a concept, *status epilepticus* can be terminated only by one of two mechanisms: (i) by the development of a more enduring depression of the excitability of the cortex, (a) "spontaneously" through repetition of the above cyclical events or (b) by the use

of drugs and other anti-convulsant agents; and (ii) by the subsidence of the tide of physiological stimuli which initiated the status epilepticus, (a) "spontaneously" or (b) by properly directed therapy. If these hypothetical considerations should prove defensible, the significance of the period of extinction to status epilepticus could be held to account similarly for the more common parallel phenomena of a single convulsive episode.

SUMMARY

1. A major convulsion is followed by a period of cortical extinction which is demonstrable (a) by the unresponsiveness of the cortex to liminal and supraliminal stimuli and (b) by the essentially isoelectric character of the cortical electrogram. The evidence from this source tends to support the contention that the phenomena which underlie convulsive seizures are qualitatively identical with those of other more typically physiological processes of excitation.

2. The restoration of cortical excitability is indicated by a slow and irregular subsidence of the isoelectric character of the electrographic tracings and by the return of cortical potentials which resemble those of the control period. Supraliminal stimuli applied to the firing point may now elicit responses resembling an aborted spontaneous seizure.

3. For a brief period following the offset of the period of extinction, the cortex in the neighborhood of the epileptogenous focus may be in an exalted state of excitability and at this time a liminal stimulus may evoke a seizure of greater violence than that which characterized the first seizure.

4. The evidence suggests a physiological account for certain phenomena observable in the clinical syndrome known as status epilepticus as well as in the more sporadic single convulsions.

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INFLUENCE OF FREQUENCY OF STIMULUS UPON RESPONSE TO HYPOTHALAMIC STIMULATION*

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FREQUENCY OF stimulation may determine the type and magnitude of the vasomotor response to electrical stimulation of an afferent nerve (1, 2). When intensity and wave form are constant, alterations in the frequency of stimulation of an afferent nerve may reverse the direction of the blood pressure response in decerebrate or spinal cats (1). The integration of the vascular responses is therefore not dependent upon the cerebral cortex, the diencephalon, or the brain stem; and it follows that the activity of the spinal neurons may be modified by the rate at which other neurons discharge onto them.

Karplus and Kreidl (3) have demonstrated that electrical stimulation of the hypothalamus could influence spinal motoneurons, causing alterations in the activity of the autonomic nervous system. In the following experiment the hypothalamus has been electrically stimulated in 13 cats. When all other characteristics of the stimulus were constant, a change in frequency often, but not invariably, affected the respiratory, vasomotor, or pupillary response.

METHOD

The hypothalamus was stimulated with bipolar electrodes inserted into the brain and manipulated with a Horsley-Clarke apparatus. The electrodes were made by cementing together two pieces of enameled nichrome wire, after the technique of Ingram *et al.* (4). The stimulator used delivered condenser discharges of a constant voltage and wave form over a frequency range of 1 to 1600 per sec. (Fig. 1). During each experiment a calibration for frequency and voltage was made from photographs of an oscillographic recording and a d.c. amplifier was used to establish that the stimulus was free of any d.c. component. The voltage (3 to 6 V.) was frequently checked during each experiment by inspection of the oscillograph recording the output of the stimulator.

The spread of current at different frequencies was tested in two cats by carefully lowering the electrodes into the facial colliculus. The position was found which permitted stimulation of the abducens nucleus without stimulating the fibers in the overlying genu of the facial nerve. At a given frequency the voltage was gradually increased until the ipsilateral eye began to close. The voltage was reduced to its original value, the frequency changed and the process repeated. The voltage had to be increased as much at a frequency of 1100 per sec. as it did at 20 per sec. This was considered adequate evidence that the spread was not increased as the frequency was increased.

Blood pressure was recorded from the femoral artery with a mercury manometer using 1 per cent sodium citrate as an anticoagulant; respiration with a pleural cannula and a tambour; and time, in 3-second intervals, was recorded with a signal magnet so adjusted that the time line represents zero blood pressure. The rectal temperature was kept between 37 and 39°C. with an electric heating pad. Ether, nembutal, or chloralose were the anesthetics used.

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The hypothalamus was explored from the preoptic area rostrally, to the mesencephalon caudally, and as far as the internal capsule laterally. The more rostral and lateral punctures were usually made first in order to avoid damage to descending connections. The electrode was lowered 1 or 2 mm at a time and a stimulus of 10 sec duration applied at each stop. When a responsive area was encountered, the electrode carrier was firmly locked in position and a number of records taken with different frequencies of stimulation. During no recording was the electrode position or the voltage of the stimulus changed. When significant responses were obtained, the electrode in some cases was lowered no further, so that the bottom of the puncture could be identified as the area of excitation.

At the termination of the experiment, the cat was perfused with saline, then with 10 per cent formalin. The brain was removed from the skull and the diencephalon prepared for histological study which permitted identification of the hypothalamic areas stimulated.

RESULTS

The results obtained are shown in the following recordings. They may be conveniently grouped into 2 categories: (1) those in which frequency changes reversed either the respiratory or blood pressure responses, and (2) those in which frequency changes caused a change only in the magnitude of response. In all the figures the number below the signal line indicates the frequency of stimuli in impulses per second.

The first group is illustrated by Fig. 2, 3 and 4. These records show that a change in the frequency of stimulation may produce a reverse in response, while repetition of the same frequency produces responses similar in type and magnitude. Figures 3 and 4 show also that when a response to a given frequency consists of two components (a rise in blood pressure during stimulation, followed by a rebound fall), a different frequency may elicit only one of these components.

The second group is illustrated by Fig. 5, 6 and 7. Here no reversals were obtained, but enormous variations in the magnitude of the response followed stimulation of the same point with different frequencies. In some cases, frequencies above or below an optimal range caused diminished responses. Stimulation at a given frequency always elicited the same response. When stimuli of different frequencies were applied in a series, neither the order of the series, the length of the interval between stimuli, nor the time allowed between one series and the next affected the characteristic response to a given frequency.

In the absence of somatic movements, the size and type of the blood pressure response could be profoundly influenced by changes in the frequency of the stimulus. In two cats, after the skeletal muscles were inactivated by curare and artificial respiration was administered, changes in visceral responses attended alterations in the frequency of excitation. In the non-curarized cat, excessive respiratory movements were often associated with a change in blood pressure, but these movements were not the sole cause of the vascular responses. They persisted in the curarized animal.

Both dilatation and constriction of the pupils during hypothalamic stimulation, and in some experiments both responses, were obtained when the electrode was kept in the same position and frequency of stimulation varied. Low frequencies (1-50 per sec) were more frequently associated

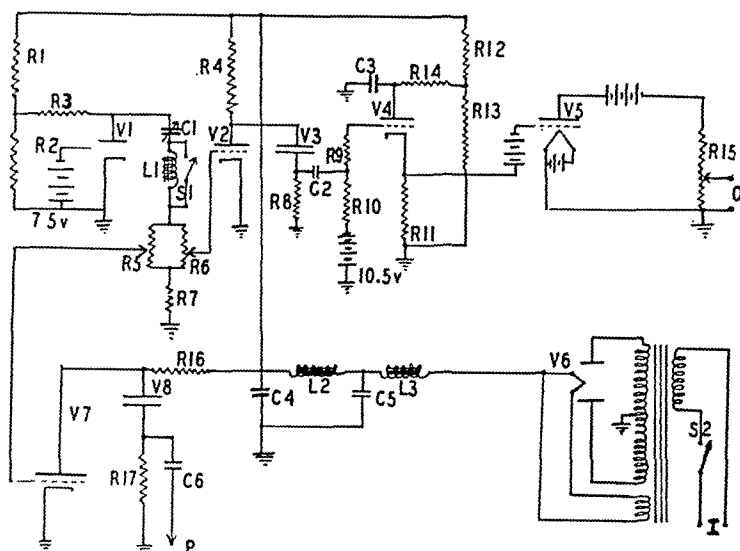


FIG. 1. Diagram of circuit of stimulator.

V1	885.	C1	Decade Condenser, .001 mf. to 1.11 mf. in .001 mf. steps.
V2	76.	C2	.05 mf.
V3, V8	2 Watt neon bulb with resistor removed from base.	C3	.003 mf.
V4	885.	C4	8 mf. electrolytic
V5	2 type 45 in parallel.	C5	8 mf. electrolytic
V6	80.		
V7	76.		
R1	100,000 Ω	L1	15 henry choke
R2	175,000 Ω	L2	20 henry choke
R3	100,000 Ω	L3	20 henry choke
R4	100,000 Ω		
R5	5,000 Ω pot.	I	110 V., 60 cycles
R6	5,000 Ω pot.	O	Output
R7	500 Ω	P	To Sweep-Oscillator Grid
R8	20,000 Ω		
R9	20,000 Ω		
R10	5,000 Ω		
R11	5,000 Ω		
R12	100,000 Ω		
R13	175,000 Ω		
R14	100,000 Ω		
R15	10,000 Ω pot.		

In this experiment, the circuit was operated with S1 closed, short circuiting L1. V1 operates as a relaxation oscillator; its frequency is determined by the value of C1. On each discharge of V1, a negative pulse appears on the grid of V2, driving the plate of V2 in a positive direction, and causing ionization of V3. At the moment of ionization of V3, a sharp positive pulse appears on the grid of V4. C3 has been previously charged to about 110 volts, but ionization of V4 has been prevented by the high negative bias on its grid. The sharp positive pulse on the grid of V4 allows C3 to discharge through it, but before discharge is complete, the high negative bias has been restored, and C3 recharges, awaiting another positive pulse on the grid of V4. V5 is biased to cut off so that no plate current flows except during the discharge of C3, when a positive pulse appears across R11. The shape and size of the output pulse depend only upon the conditions existing on V4 when a pulse is tripped. Adjustment of C1 varies the frequency of the oscillator V1, and thus varies the repetition



FIG 2 Four records showing stimulation of the same point in the hypothalamus a) Higher frequency, applied first, caused a rise in blood pressure and acceleration of respiration, lower frequency caused a fall in blood pressure and slowing of respiration b) Sequence of stimuli reversed, and responses reversed The electrodes were in the central grey at the rostral end of the midbrain

rate of the tripping pulses The output pulses are not strictly independent of frequency, since the higher the frequency, the earlier in the charging cycle of C3 tripping occurs However, the charge on C3 is an exponential function of time and, over the range of frequencies used, tripping occurs near the asymptote, so that the variation of pulse size with frequency is very small The difference in pulse size from one end of the range to the other could not be detected on oscillographic analysis

The inductance L1 and vacuum tubes V2, V3, V7, and V8 are not essential to the operation of the circuit as used in this experiment, they are used in other applications to secure desired time relations between the stimulus and the tripping of a 'single sweep' circuit so that action potentials resulting from the stimuli may be observed as a standing wave L1 broadens the discharge pulse of V1 so that it approximates the positive half of a sine wave The points on this wave at which V3 and V8 ionize may be varied by adjusting R5 and R6, and a slight time lag between the start of the sweep and the application of the stimulus may thus be obtained Where this feature is not required, the circuit of V1 could be arranged to deliver a positive pulse on discharge, and the pulse could be applied to the grid of V4 through a suitable resistance capacity network without intermediate vacuum tubes

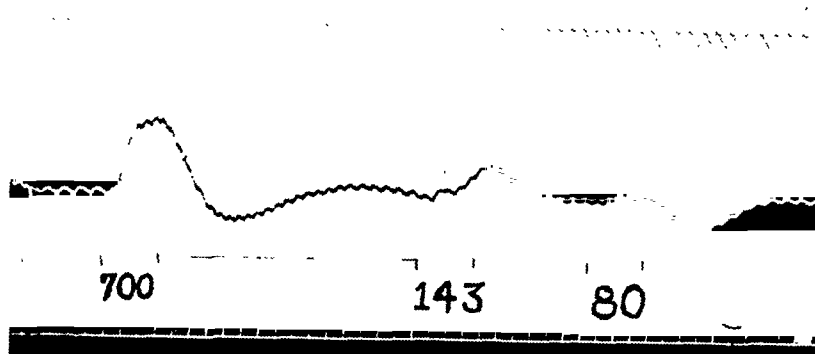


FIG. 3. First stimulus (highest frequency) caused a slight increase in the rate of respiration and a sharp rise in blood pressure, which was followed by a fall after the end of stimulation. Second stimulus, at a much lower frequency, caused only a slight, delayed rise in blood pressure; and the third stimulus, at a still lower frequency, caused no apparent disturbance during excitation, but was followed by a rebound fall in blood pressure. The electrodes were in the anterior hypothalamic area just caudal to the optic chiasm.

with pupillary constriction, while high frequencies (50–1600 per sec.) usually caused the pupils to dilate. The nature of the response appeared to depend upon the frequency of stimulation, rather than upon the position of the electrode in the hypothalamus. If the electrode were in the optic tract or the oculomotor nerve, changes in the stimulating frequency caused only a change in the size of the pupillary response, without once altering its sign.

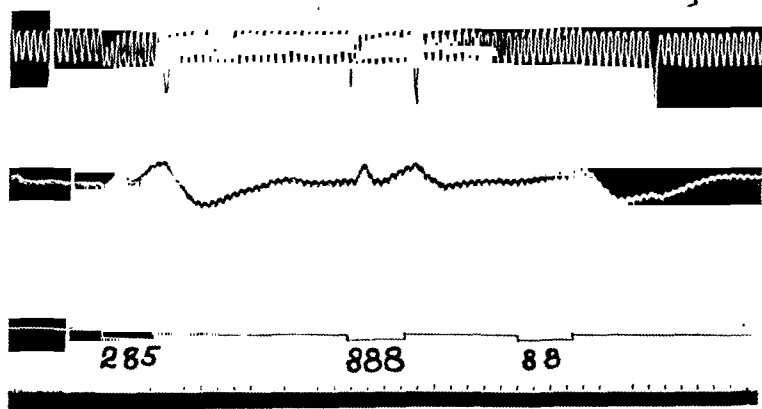


FIG. 4. A record similar to Fig. 3, except that the highest frequency shows only the pressor response. Since this is a part of a long continuous record, reference points, equidistant from the starting points, have been added in white ink. The electrodes were in the posterior hypothalamic nucleus.

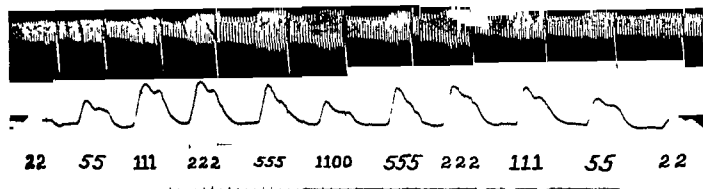


FIG. 5. Consecutive records taken without stopping the kymograph, showing variation in magnitude of pressor and respiratory responses with different frequencies. Optimum frequency range for pressor response is between 55 and 555 impulses per sec. Repetition of same frequency produces similar responses. The electrodes were in the rostral end of the ventromedial hypothalamic nucleus.

DISCUSSION

Pressor and depressor responses were obtained from both the anterior and posterior hypothalamus; the nature of the response was often dependent upon the frequency of the stimulus. Bronk, Pitts and Larrabee (5) have observed a fall in blood pressure during hypothalamic stimulation of a frequency of 2 per sec. associated with a cessation of electrical activity in an efferent sympathetic nerve, the inferior cardiac. Stimulation of the same hypothalamic area at the rate of 20 per sec. caused an increase in the blood pressure and an increased sympathetic activity. If central or peripheral stimulation may inhibit the vasopressor apparatus, it seems improper to conclude from a fall in blood pressure that a "parasympathetic" nerve or center has been excited. Since dilatation of the pupil may occur reflexly in the absence of any innervation from the thoraco-lumbar division of the autonomic system (6, 7, 8), a widening of the pupil during hypothalamic stimulation may be an expression of decreased activity of the oculomotor

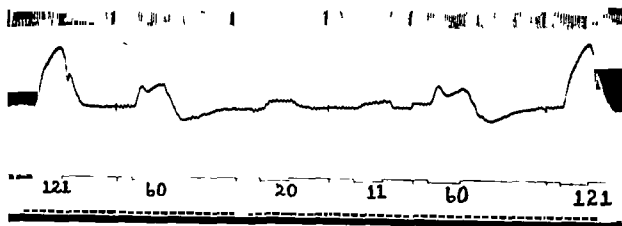


FIG. 6. Compare first stimulus with last, and second with fifth. Discontinuous record. The electrodes were in the anterior portion of the hypothalamus near the rostral end of the dorsal hypothalamic area.

nerve. In these experiments one and the same area produced both "sympathetic" and "parasympathetic" responses when activated with stimuli of constant voltage and wave form, but of different frequencies.

The importance of the frequency of stimulation in determining the response is not peculiar to the responses of the visceral effectors. Somatic responses are just as profoundly affected; threshold, facilitation, fatigue, inhibition, and type and magnitude of postural responses, whether elicited from the cerebral cortex or the diencephalon, were all influenced by the fre-

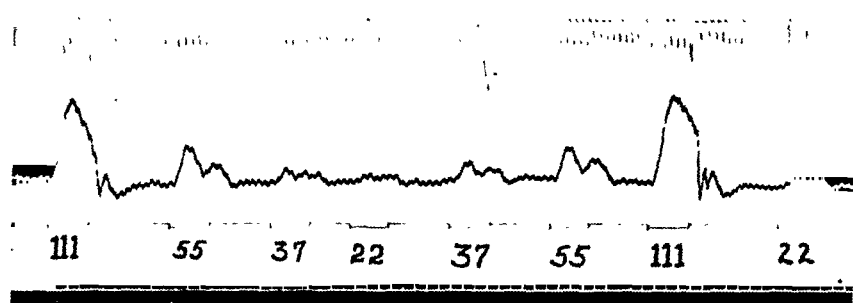


FIG. 7. Similar to Fig. 6, but continuous. The area stimulated was dorsal to the optic chiasm, between the nucleus ovoideus and the supraoptic (tangential) nucleus.

quency of excitation (9). The only somatic movements graphically recorded in the present experiments were respiratory. In almost every case the rate and depth of respiration changed when the frequency of the stimulus changed. In Fig. 2, a low rate of stimulation decreased the respiratory rate; a higher frequency of stimulation caused an acceleration of respiration.

The responses to stimulation of some areas in the hypothalamus could be reversed in sign by altering the frequency of the stimulus (Fig. 2); other areas yielded responses which could be altered only in magnitude (Fig. 5). Reversals of response in this series of experiments occurred often enough to raise a question about strict localization within the hypothalamus of areas which are designated as pressor, depressor, pupillary dilator, or pupillary constrictor.

SUMMARY AND CONCLUSIONS

1. In most cases the magnitude of the pressor and respiratory responses increased with frequencies up to several hundred per second. Beyond this optimum, further increases in frequency caused a progressive diminution of the response. This optimum varied from one part of the hypothalamus to another and from cat to cat.

2. Neither facilitation nor fatigue was responsible for the changes in response. An entire series of responses to stimulation of one area could be repeated immediately or after half an hour and each frequency would elicit

its characteristic response The sequence in which the stimuli were applied in a series did not affect the response

3 Differences in spread of current at different frequencies do not account for the change of response

4 In some instances, changes of frequency altered the character of the response

5 Alterations in the frequency of stimulation caused changes in the visceral responses in cats in which the skeletal muscle had been paralyzed by curare

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EXPERIMENTAL STUDY OF GASTRIC ACTIVITY RELEASED FROM CORTICAL CONTROL

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NUMEROUS clinical and experimental studies have dealt with the influence of the cerebral cortex upon the activity of smooth muscle. Fulton (1938) has made an excellent review of the subject. In the recent symposium by Langworthy *et al.* (1940) on the physiology of micturition, the cerebral cortex was considered in relation to vesical activity. However, few viscera besides the bladder are so readily adaptable to investigative procedures.

Although easily accessible, the gastro-intestinal tract, particularly the stomach, is known to be regulated by the interaction of a great complexity of mechanical, chemical and neurogenic factors. Nevertheless, certain clinical observations have suggested an influence by the cerebral cortex over gastro-intestinal movements. Stomach complaints develop frequently in anxiety states and other disorders at higher levels of integration. Watts and Frazier (1935) asserted that the abdominal aura often preceding epileptic convulsions coincides with abnormal gastro-intestinal motility. Robinson (1939) concluded from the radiologic examination of 100 patients that there is no characteristic gastro-intestinal pathology in epilepsy but did not study gastric motility in relation to convulsions. Definite gastric phenomena, particularly the syndrome of morbid hunger and restlessness as described by Levin (1936), are known to occur in relation to brain tumors, cerebral degenerative disease, trauma and other processes damaging the frontal cortex. Levin (1935) discussed the same condition associated with cortical diplegia in children.

Experimental data derived from stimulation and ablation procedures have further indicated a control of gastro-intestinal activity by the cerebral motor and premotor cortices. Bochefontaine (1876) first produced gastric contraction, pyloric relaxation and increased peristalsis in the dog's small intestine and colon by stimulating the sigmoid gyrus. His results have been corroborated by recent workers, particularly Watts (1935) who gave evidence that cortical representation for the gastro-intestinal tract contains both excitatory and inhibitory components. Watts and Fulton (1935) delimited the more excitable cortical foci to area 6 in monkeys. They (1934) also showed that bilateral partial or complete ablation of the frontal lobes caused morbid hunger and occasionally intussusception. Mettler *et al.* (1936) used roentgenographic methods to demonstrate disturbances in gastro-intestinal function in the cat after localized ablations of cerebral cortex.

Although previous clinical and experimental evidence is significant and the nervous regulation of gastro-intestinal activity has been investigated by

various methods, the influence of the cerebral cortex on gastric motility has never been demonstrated graphically. Langworthy and Kolb (1935) produced a condition simulating pseudobulbar palsy in cats by removing both cerebral motor cortices. Their animals subsequently developed general over-activity and ravenous appetites. The present paper entails a graphic study of the alterations in gastric motility in such preparations.

METHOD

A simple yet constant and reliable recording device was utilized (Fig. 1). A light rubber balloon, fastened over the tip of a small soft rubber catheter (14 French), was introduced through the mouth into the stomach. The catheter was connected to a water reservoir in

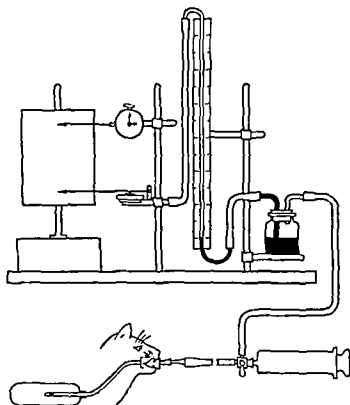


FIG. 1. Diagram of the gastrometric apparatus.

such a way that fluctuations of air pressure in the stomach balloon would register on the metric scale of a water manometer attached to the reservoir. Corresponding variations in the air column over the manometer fluid were recorded by tambour on a kymograph. Time marks and pressure-volume values were included on the tracings. The stomach was filled with air* in 20 cc. increments at appropriate intervals. This was accomplished by means of a syringe and 3-way stop-cock inserted in the system between catheter and water reservoir. Since the balloon filled the entire viscus, the pressure gradients as recorded graphically represented a constantly changing summation of all smooth muscle activity in the stomach wall. Hence it was necessary to recognize total responses rather than individual peristaltic movements.

* In their studies on micturition, Langworthy and his coworkers used water to distend the bladder. This method, however, was not suitable for gastric activity because the weight of the water-filled balloon method but its weight on the stomach wall dampened the recording process. Air, acting as a pressure-transmitting medium against a water column, was found to afford maximal sensitivity to minute changes in intragastric pressure.

In order to obtain satisfactory records, it was found convenient to immobilize the animals. Those forcibly restrained while conscious struggled so that the tracings were unsatisfactory. Anesthesia with ether or nembutal either subdued or completely abolished stomach activity. Finally, bulbocapnine† produced a cataleptic-like state in the conscious experimental animal without interfering significantly with peristalsis. This drug, which was used during all gastrometric readings in the present study, had been employed previously by Kolb and Langworthy (1938) in studies on micturition and is admirably suited to such purposes. About 100 mg. of bulbocapnine subcutaneously were sufficient to quiet a 2 to

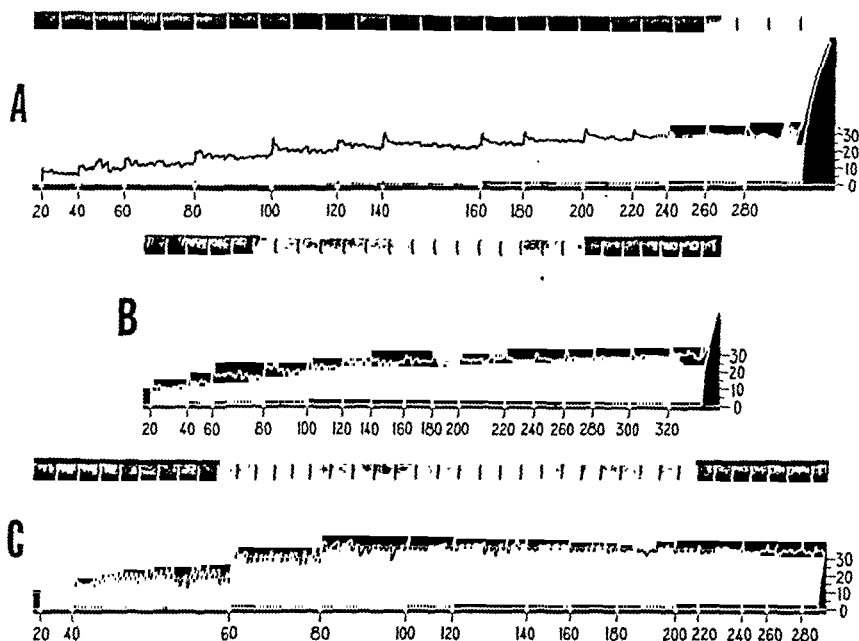


FIG. 2. Kymographic tracings of stomach activity in one of our preparations before and after successive removal of the motor cortices. The upper record (A) was made from the normal animal, the middle (B) followed ablation of the motor cortex on one side and the lower (C) was made after removal of the second cortex. Pressure values in centimeters of water are placed at the end of each record. Volume is indicated in cubic centimeters of air where each increment was introduced. Time is marked in five-second intervals accentuated every minute.

3 kg. cat. Some animals tended to cry while under the influence of the drug and were best studied blindfolded in absolute quiet.

Records were made from the normal animal and at appropriate intervals after unilateral and bilateral removal of the motor cortices. All operations were performed aseptically under nembutal anesthesia. Full recovery from shock and debilitation was permitted between operations and before gastrometric readings.

EXPERIMENTAL FINDINGS

Studies were made upon 6 cats; representative records and composite results are given. Figure 2 consists of a series of typical tracings from one of

† Bulbocapnine was obtained through the kindness of the Merck Co., Inc., Rahway, N. J.

our animals. Volume increments are designated on the baseline in cubic centimeters of air and pressure values are indicated at the end of the records in centimeters of water. Time is marked in 5-second intervals accentuated every minute.

The first graph (A) was made from a normal animal. It will be seen that the intragastric pressure, which rose to 5 cm. of water after the first 20 cc. increment, increased quickly to 15 cm. during the subsequent four increments. The pressure then continued to rise slowly until the limits of stretch were reached at 260 cc. of air and 28 cm. of water. After the introduction of the next 20 cc. of air the pressure fell slightly. A minute later the cat drooled, retched and vomited. Waves of gastric contraction occurred throughout the record but were most intense in the early stages of filling and hardly perceptible toward the limits of stretch. The waves were irregular, averaged 3 or 4 to the minute and never exceeded 5 cm. of water in amplitude. The stomach accommodated promptly to the sudden stretch of each added increment of air.

The second tracing (B) was obtained at a suitable period after the removal of one motor cortex. It shows a slightly earlier and higher rise of intragastric pressure than the first record. After 100 cc. of air had been introduced into the stomach, the pressure remained at 20–25 cm. of water until filling was nearly complete. When the last increment, totalling 320 cc., was injected the pressure rose to 30 cm., but, as before, it fell slightly within the next minute and the animal retched and vomited. An occasional increase in the amplitude of gastric contractions was evident with no essential change in their frequency or duration. In some instances, slowness in accommodation by the stomach wall to added increments suggested a response to stretch. This could be seen especially after the third and fourth additions of air.

The third record (C) followed removal of the second motor cortex. The cat had recovered fully from the operation with characteristic overactivity and ravenous appetite. As filling progressed, tonus in the stomach wall was greater than ever before, and past the 100 cc. level the pressure remained around 35 cm. of water. The experiment was concluded at 280 cc. although no retching occurred. A marked increase in the amplitude of gastric contractions was evident soon after the second increment when a distinct elevation of tone accompanied the onset of activity. The waves fluctuated as much as 12 to 14 cm. of water, and this activity persisted to the end of the record with only slight reduction in amplitude. There was no change in the form or frequency of the waves.

In Fig. 3, the top tracing (A) was made from an intact animal and the middle tracing (B) from the same animal after removal of both motor cortices. The first record is similar to that in Fig. 2 except for a stomach capacity of 500 cc. and more marked peristaltic activity earlier in filling. After operation, the tone baseline during accommodation was distinctly higher with increased amplitude and persistence of peristalsis. Most striking

was the delayed relaxation following each addition of air. In some instances, as after increments 80, 120, 140, 160 and 180, the tone baseline dropped momentarily to regain its former level within the next 30-40 sec.

The lower three tracings in Fig. 3 represent activity in the oesophagus of one of our preparations. After the gastrometric reading, the stomach balloon

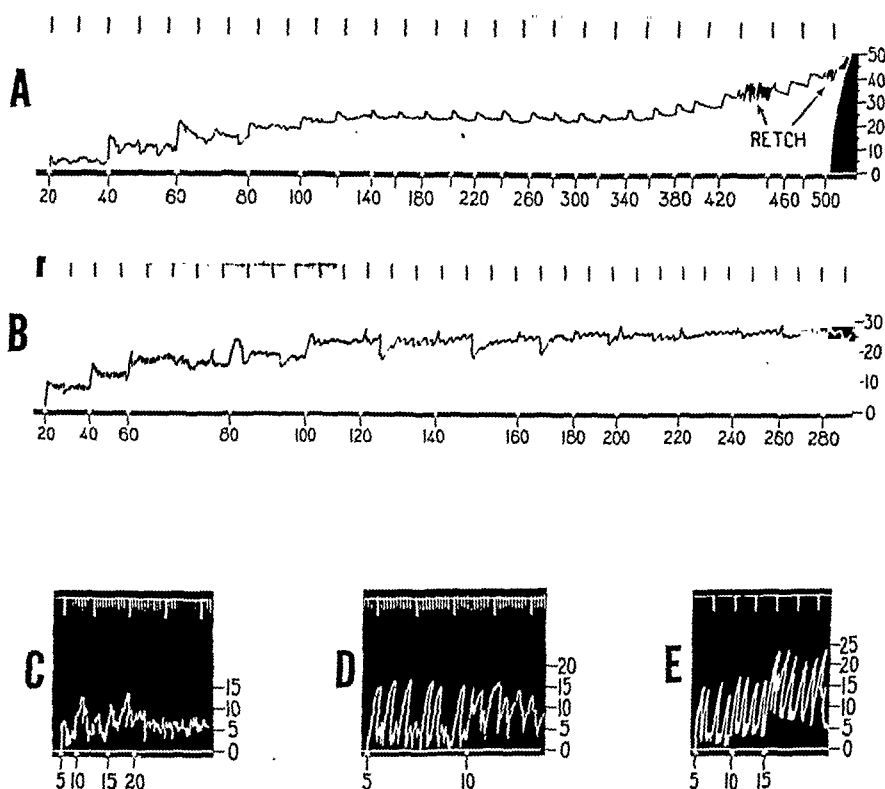


FIG. 3. Kymographic tracings of stomach and oesophageal activity in another of our preparations before and after successive removal of the motor cortices. The upper record (A) was made from the normal animal. The middle tracing (B) was obtained after removal of both motor cortices and shows unusual types of wave activity. The lower three tracings were made from the same animal by pulling the stomach balloon into the oesophagus. The first record (C) was made from the normal animal, the second (D) after ablation of the motor cortex on one side and the third (E) following removal of the second cortex. Pressure, volume and time values are indicated as in Fig. 2.

was deflated, pulled into the oesophagus and reinflated with air in 5 cc. increments. The first record (C), made from the normal animal, showed irregular contraction waves averaging 2 or 3 per min. They never exceeded 8-10 cm. of water in amplitude and disappeared after 20 cc. of air had been injected. The tone baseline averaged 5 cm. of water. In the second record (D), obtained after removal of one motor cortex, contractions were more regular and increased in amplitude to 10-12 cm. with no change in frequency or baseline. The third graph (E), made following ablation of the second cor-

tex, demonstrated even greater oesophageal contractions of 12-15 cm. recurring regularly at the same rate throughout filling. There was also a slight baseline elevation to 8 cm. of water after the third increment. During all oesophageal readings, considerable traction on the catheter was required to prevent contraction waves from forcing the balloon into the stomach.

Arranged similar to the records in Fig. 2, a diagrammatic representation of the changes in gastric activity occurring after successive removal of the motor cortices is given in Fig. 4. The schematic tracings are patterned after an analysis of studies on 6 cats in an effort to illustrate the increase in tone

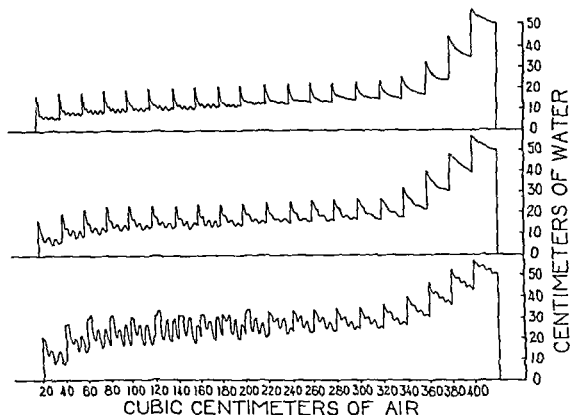


FIG 4 A diagrammatic representation of the changes produced in gastric activity by successive ablation of the cerebral motor cortices. The schematic tracings are patterned after an analysis of our studies on six cats in an effort to illustrate changes which occur in tone, wave activity and response to sudden stretch. The upper diagram represents these factors in the normal animal, the middle illustrates changes following removal of one motor cortex and the lower shows the result of ablation of the second cortex.

level, the greater amplitude and persistency of gastric contractions and the slowness of accommodation to added increments which took place in our preparations.

The normal stomach responds to stretch by active reflex relaxation to an appropriate tone level. The stomachs of these animals varied considerably in tone, motility and response to stretch. The typical pressure curve rose abruptly during initial filling and slowly or not at all throughout accommodation even though the stomach volume increased steadily. The curve remained fairly constant for each animal and was marked by small irregular waves most pronounced early in filling and inclined to disappear during accommodation or at the limits of distension. At the limit of smooth muscle

elongation, the pressure curve rose quickly again as the connective tissue network limited further stretching of the stomach wall. This point was usually ushered in by drooling and terminated by vomiting. There were occasional minimal responses to sudden stretch characterized by a brief rise in pressure immediately after the introduction of an increment of air. The initial steep rise in pressure usually leveled after the introduction of 60 to 80 cc. of air. From this point on the pressure averaged 10 to 15 cm. and wave fluctuations 1 to 3 cm. of water. Since the pressure rose rapidly at the limits of distension, readings were discontinued after the pressure reached 50 cm. of water. Stomach volumes varied from 300 to 500 cc. at this level.

Changes in gastric activity were evident in some instances, as described above, after the removal of one cortex. In general, however, they were inconstant.

Following removal of the second cortex, most of the recovered animals exhibited restlessness and ate greedily. Stomach records made at this time revealed a distinctly altered wave pattern marked by greater amplitude, regularity and persistence of excursions. Tone in particular was increased throughout filling, especially at the height of wave activity. This usually began early in the period of expansion and continued well into the limits of distension. Distinct and consistent responses to sudden stretch followed each added increment. Pressure rose more quickly during initial expansion and averaged 20 to 25 cm. of water during accommodation. Wave fluctuations varied between 10 and 20 cm. and at times showed a tendency to periodicity. Stretch responses caused additional temporary and sometimes prolonged pressure elevations of 3 to 5 cm. No changes in stomach volume were evident.

After removal of both cerebral motor cortices there was less tendency to retch and vomit at the limits of distension, but drooling would occur as usual. The animal would often remain quiet and sometimes sleep in a plaster cast during the reading without the administration of any drug. Under these circumstances, periods of skeletal muscle activity manifested as running movements of the legs would develop at the onset of powerful waves of gastric contraction. The cat would become quiet again as the waves subsided. This phenomenon suggests a correlation between the increased spontaneous activity produced by frontal lobe lesions, studied quantitatively by Langworthy and Richter (1939), and a state of gastro-intestinal hypermotility.

DISCUSSION

Nervous control of smooth muscle activity in different hollow viscera varies in accordance with the demands placed upon them. It has been shown that the bladder stores quantities of urine at relatively low pressures, whereas eventually a sustained contraction of the vesical muscle empties the organ completely. The stomach in its entirety has two functions, the storage of food by appropriate relaxation and the trituration of food by

rhythmic peristaltic action. The latter also causes progressive emptying when it is properly synchronized with the activity of the pyloric musculature. These functions are coordinated reflexly with the degree of filling, the character of the gastric contents and outside influences mediated through control by the central nervous system.

Our experiments record graphically definite alterations in gastric tone and motility following ablation of the cerebral motor cortices in the cat. The demonstrable production of greater persistency, consistency and strength of stomach contractions and increased tone throughout distension is unmistakable evidence of release from a regulating influence by the motor cortex. The apparent development of a marked stretch reflex with delayed relaxation of the stomach wall after sudden distension is confirmatory.

The results of this study correlate in some degree with earlier studies by Langworthy and Hesser (1936) on micturition released from cerebral control. The effect of removal of the cerebral motor cortices as an increased response of the vesical muscle to stretch was not so well demonstrated in their preparations. This was probably due to a retarding effect by the nembutal used during cystometric readings. However, it was clear that the vesical capacity was reduced after extirpation of one motor cortex and further decreased following removal of the second cortex. The bladder practically lost its ability to accommodate increasing quantities of fluid at low pressures. This development apparently resulted from increased tone in the bladder wall which raised the pressure to the emptying point more quickly past a weakened external sphincter.

Although the method employed in the present study could not demonstrate the emptying point of the stomach, the increase in tone and peristaltic activity following ablation of the motor cortices might be considered, by analogy, conducive to faster emptying and a smaller stomach capacity. Watts (1935) reached similar conclusions in his studies on monkeys. Increased peristalsis would certainly result in a quickening of hunger stimuli which, already lacking certain cerebral retardation, would aggravate the manifestations of great restlessness and ravenous appetite.

Mosso and Pellacani (1882) first demonstrated the peculiar ability of the urinary bladder to hold different quantities of fluid at approximately the same pressure. We wish to emphasize the similar characteristics of the stomach filled progressively with air as shown in the present study.

Of interest was the tendency of the normal as well as the decorticate animals to drool, retch and vomit at the limits of stretch. That the inclination to the latter two was somewhat diminished after removal of the motor cortices would seem to indicate a cerebral influence on the initiation of the reflex act of vomiting as it is integrated at lower levels. The afferent stimuli responsible for the initiation of retching and vomiting seem to have been derived from the state of tension developed in the confining connective tissue network of the stomach wall at the limit of stretch.

It is significant to note that changes similar to those in stomach wall

activity took place in the oesophagus as well. These developed particularly as increased wave amplitude, regularity and persistency and as a slight elevation of the tone baseline. It was apparent, however, that oesophageal activity was of a specialized type designed to propel food into the stomach. Powerful individual contraction waves progressed down the oesophagus at more or less regular intervals to cause strong traction on the expanded balloon. This was frequently forced into the stomach in spite of efforts to hold it back. Since the balloon extended almost the entire length of the oesophagus, it was impossible to distinguish smooth muscle activity in the lower oesophagus from that of striated muscle in the upper oesophagus. Nevertheless, these demonstrations not only serve to indicate a similarity in the innervation of the smooth muscle of oesophagus and stomach but also emphasize a basic responsiveness of smooth muscle to alteration in its nervous regulation regardless of its special function as part of an organ system.

Our experiments suggest, then, that the smooth muscle of the stomach and oesophagus is controlled by reflex pathways in the nervous system similar to those which control tone and contraction in the vesical muscle. It is probable that the tone and contraction are dependent primarily upon afferent stretch stimuli arising in the muscle itself. This has already been shown to hold true for the bladder. The stretch reflex in bladder and stomach alike is not always evident in the normal animal but can be demonstrated easily after cortical control is removed.

Smooth muscle is represented functionally at higher levels of integration and not necessarily in terms of sympathetic or parasympathetic influence. The cerebral cortex certainly mediates the power to initiate micturition or to suppress vesical contractions and so postpone micturition. In studies of the bladder, it was shown graphically that certain disorders of function are dependent upon organic disease of the nervous system. Often it was possible to postulate the anatomical lesion that produced the abnormality. It is probable that similar criteria can be established for the stomach.

Micturition is always initiated by contraction of the vesical muscle, whereupon the internal vesical orifice is opened mechanically. Many abnormalities of micturition follow disturbances in the force and duration of contraction in the musculature of the vesical wall and not derangements in the sphincters themselves. The term "sphincter disturbance" is, therefore, incorrect.

It is probable that similar conclusions can be drawn concerning the cardiac and pyloric sphincters of the stomach. It has become clear that relaxation of the pyloric sphincter is related directly to the strength of peristaltic waves in the antrum. The manifestations of "cardiospasm" or achalasia of the oesophagus are perhaps dependent not on failure of the cardiac sphincter to relax but primarily on a defect in the power of the smooth muscle in the lower oesophagus to propel food into the stomach.

CONCLUSIONS

Utilizing a balloon-tambour air-water system, graphic studies of stomach activity in the cat were made before and after successive removal of the cerebral motor cortices. Definite alterations in gastric activity followed ablation of the motor cortices and were demonstrable as greater persistency, consistency and strength of stomach contractions along with increased tone throughout distension. This was interpreted as evidence of release from a regulating influence by the motor cortex. A marked stretch reflex with delayed relaxation of the stomach wall after sudden distension was also apparent. In the oesophagus similar changes occurred after operation as increased wave amplitude, regularity and persistency and as an elevation of tone. An attempt was made to correlate the results of the present study with those made earlier on micturition released from cerebral control. It was suggested that the smooth muscle of the stomach and oesophagus is controlled by reflex pathways in the nervous system similar to those which control tone and contraction in the vesical muscle.

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PREFATORY NOTE

THE FOUR PAPERS included in this number were in preparation for publication at the time of Dr. Dusser de Barenne's death on June 9, 1940. His colleagues, Drs McCulloch and Garol, who were collaborating with him in his investigations, have completed the work and prepared the papers for press. It seemed to the Editors of the *Journal* that the papers, if published together, would constitute a fitting tribute to the memory of their former colleague. The papers are therefore being issued together in this special number which has been financed by private subscription.

A bibliography of Dr. Dusser de Barenne's scientific writings was appended to the appreciation which appeared in the *Journal* for July 1940 (pp. 283-292). To complete this list we may now add the following titles which have appeared since that time:

- DUSSER de BARENNE, J G, GAROL, H W and McCULLOCH, W. S. Sensory cortex of chimpanzee *J Neurophysiol*, 1940, 3 469-485
- MURPHY, J P and DUSSER de BARENNE, J G Thermocoagulation of motor cortex exclusive of its sixth layer *J Neurophysiol*, 1941, 4 147-152
- DUSSER de BARENNE, J G, MARSHALL, C, NIMS, L F and STONE, W E. The response of the cerebral cortex to local application of strychnine nitrate *Amer J Physiol*, 1941, 132 776-780
- DUSSER de BARENNE, J G, GAROL, H W, and McCULLOCH, W S The "motor" cortex of the chimpanzee *J Neurophysiol*, 1941, 4 287-303
- DUSSER de BARENNE, J G, and McCULLOCH, W S Functional interdependence of sensory cortex and thalamus *J Neurophysiol*, 1941, 4 304-310
- DUSSER de BARENNE, J G, and McCULLOCH, W S Suppression of motor response obtained from area 4 by stimulation of area 4s *J. Neurophysiol*, 1941, 4 311-323
- DUSSER de BARENNE, J G, GAROL, H W, and McCULLOCH, W S Functional organization of sensory and adjacent cortex *J Neurophysiol*, 1941, 4 324-330

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- DUSSER de BARENNE, J G A method for uniform sectioning of brain, *J Tech Methods and Bull internat Ass med Museums*
- DUSSER de BARENNE, J G, GAROL, H W, and McCULLOCH, W. S Physiological neuroanography of the cortico striatal connections *Res Publ Ass nerv ment Dis*

THE EDITORS



THE "MOTOR" CORTEX OF THE CHIMPANZEE*

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(Received for publication April 22, 1941)

INTRODUCTION

IN PREVIOUS papers dealing with the sensory cortex of the chimpanzee^{1 2 29 37} we have mentioned that in the animals upon which those experiments were performed an exploration of motor response to electrical stimulation of the cerebral cortex preceded each investigation of the location, subdivision or functional organization of the sensory cortex. These explorations differ in four respects from those reported by other investigators.^{4 54 55 56 59 61} (i) The subsequent studies on the sensory cortex necessitated the use of a head holder which so fixed the jaws and immobilized the head that the tongue could never be observed and contractions of neck musculature were manifest only to palpation. (ii) The chimpanzees were under Dial narcosis of such a depth that there was usually much tension of the extensor and frequently moderate tension of the flexor muscles. (iii) The carefully controlled electrical stimulation was usually by very long pulses, often at extremely low frequencies. (iv) The area stimulated was not confined to the precentral convolution but extended over the entire exposed hemisphere, i.e., from in front of the "motor" eye field to behind the sulcus lunatus and from as far medial as could be reached with the stimulating electrodes, without displacing the brain, to the fissura Sylvii laterally. In this paper are recorded all phenomena obtained by stimulation anywhere in this extensive area.

METHODS

Nine chimpanzees (*Pan satyrus*) 2½ to 3½ years old were used altogether. The animals were given 0.35 to 0.45 cc Dial§ per kg body weight, ½ intraperitoneally, ½ intramuscularly. This produced full anaesthesia without abolishing muscular tension. The narcotized animal was placed on a board with the hind quarters elevated to maintain good cerebral circulation and with the head fixed to the board by one bar over and behind the lower incisors and a second behind the occiput.

One hemisphere was then exposed by turning down a large osteoplastic flap. In opening the dura mater special care was taken not to injure the cerebral veins passing to the dural lacunae. Photographs from several angles were taken and printed and the location of each stimulation was recorded on these as well as on drawings of twice life-size.

In most of the experiments the contralateral arm and leg were then suspended by heavy rubber bands so disposed as to allow freedom of movement without compromising circulation. In these cases the motions were noted by two or more observers and any change in muscular tension was controlled by palpation and passive movement of the part affected.

In the earliest of these experiments an ordinary thyatron stimulator (after Schmitt⁷⁴) was employed. Thanks to Craig Goodwin, electronic engineer for this laboratory, it was later replaced by an instrument of greater flexibility.⁵² This permits independent control

* Aided by a grant from the John and Mary R. Markle Foundation.

† Deceased June 9, 1940.

‡ National Research Fellow 1940-1941.

§ We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

Although in seven of the chimpanzees both hemispheres were stimulated most of the findings reported here were obtained from the first hemisphere, for, while the second was usually in good condition when exposed, the animal had then been on the table for a matter of a day or more, the threshold had gone up and responses were less discrete. Thus only a rough map of it was warranted.

FINDINGS

The time relations for primary facilitation (*i.e.*, the fall in threshold, diminution of latency or increase in amplitude induced by antecedent stimulation of one and the same focus) and extinction (*i.e.*, the rise in threshold, increase in latency or decrease in amplitude induced by antecedent stimulation of one and the same focus) and their dependence upon the parameters of stimulation are grossly similar to those found in the macaque (see Fig. 2a). The response, however, is more complicated in character and at values of stimulation near threshold the latency is much less.

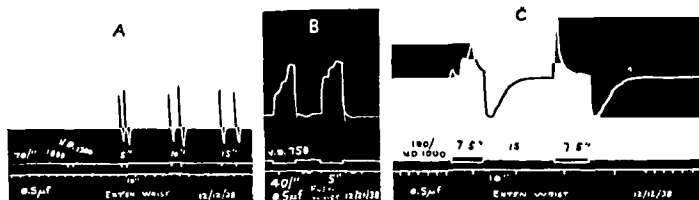


FIG 2 Isotonic recording via air tambours. Monopolar stimulation $0.5\mu f$. A Shows facilitation with intervals of 10 sec., with partial extinction at 15 seconds. B Shows triphasic response with more marked facilitation of the earlier components. C Triphasic response showing facilitation of first component with extinction of subsequent components. In each case the response is followed by a contraction of the antagonistic muscles.

If the stimulation is continued for 2 to 2.5 seconds the response usually consists of 3 components. The first of these occurs, even at threshold values, with a latency too small to detect on the kymographic record. The second component appears at least a second later and the third about a second later still. With weak stimulation the second is larger than the first and the third is larger than the second. With stronger stimulation the first is disproportionately larger. No fourth component appears even when stimulation is prolonged to 7.5 sec. (see Fig. 2b). With respect to facilitation and extinction the components behave differently. As extinction is enhanced (by increasing the frequency or the duration of stimulation or by increasing the interval) the later components, particularly the third, diminish or disappear, while the first still exhibits facilitation (see Fig. 2c).

Motor bands—The lowest threshold was found immediately anterior to the fissura centralis, *i.e.*, Band V (see Fig. 3). Immediately frontal to this band, *i.e.*, in Band IV, the threshold was definitely higher. (Both of these regions gave highly specific movements.) Even in these regions the thresh-

hold was higher in the sectors for neck and truck than in those for face, arm and leg.

Band II gave mixed motor responses of leg and arm, of arm, eyes and face, and even of leg, arm and face in its most antero-medial portion. When the animal was lightly narcotized and in good condition these responses were readily elicited, whereas when it was deeply narcotized they could be obtained only after facilitation or prolonged stimulation. In the latter case they were frequently followed by motor after-discharge.

Approximately the anterior half of the postcentral convolution, Band VI, gave discrete movements which were different from those elicited from foci immediately precentral to them and that without altering the stimulus. This presumably eliminates spread of current as the efficient cause of these responses.

T. Graham Brown defines secondary facilitation: "The facilitation of a cortical point therefore seems to raise the excitability of a wide area of the surrounding cortex for the reaction evoked by the stimulation of that point."¹¹ (see p. 122), 1. 20 By secondary facilitation, starting from either the pre- or postcentral gyrus, movements were obtained from Bands VIII and IX providing there was appreciable muscular tension in the extremity involved. This was repeatedly done only in the arm region where our investigations were most extensive.

Suppressor bands^{30 34 39 51 57}.—Stimulation of Band I, III, VII or XI, even with 3 or 4 times the voltage which elicited response from adjacent motor foci, failed to elicit movement. Yet it had the following effects.

First, it suppressed motor response to electrical stimulation,^{26 28} i.e., after stimulation of any of these bands stimulation of a motor focus with a stimulus which previously elicited a motor response now failed to do so.

Second, it suppressed motor after-discharge following cortical stimulation, i.e., during several seconds of stimulation of any one of these bands the clonic contractions initiated by stimulation of Band II, IV, V or VI were held in abeyance. This could be repeated several times during a single motor after-discharge. More prolonged stimulation of these bands terminated it and prevented its reappearance.

Third, it relaxed existing muscular contraction, i.e., stimulation of any of these bands caused a disappearance of resistance to passive motion, a softening of muscle bellies as judged by palpation, and a falling in response to gravity of an extremity previously supported only by its muscular tension.

In order to make clear the nature of these explorations and the type of

sensory band of Elliot Smith. *Below.* Diagrammatical representation of the directed functional (and anatomical) relations between the various cortical bands of the arm-subdivision of the sensory cortex found in these experiments and also those of Bands I and XI adjacent to but outside of the sensory cortex. Anterior and posterior borders are the limits of the sensory cortex. The suppression of the ECG of various bands upon stimulation of Bands I, III, VII and XI is indicated thus: — = definite "firing" but uncertainty as to "fired."

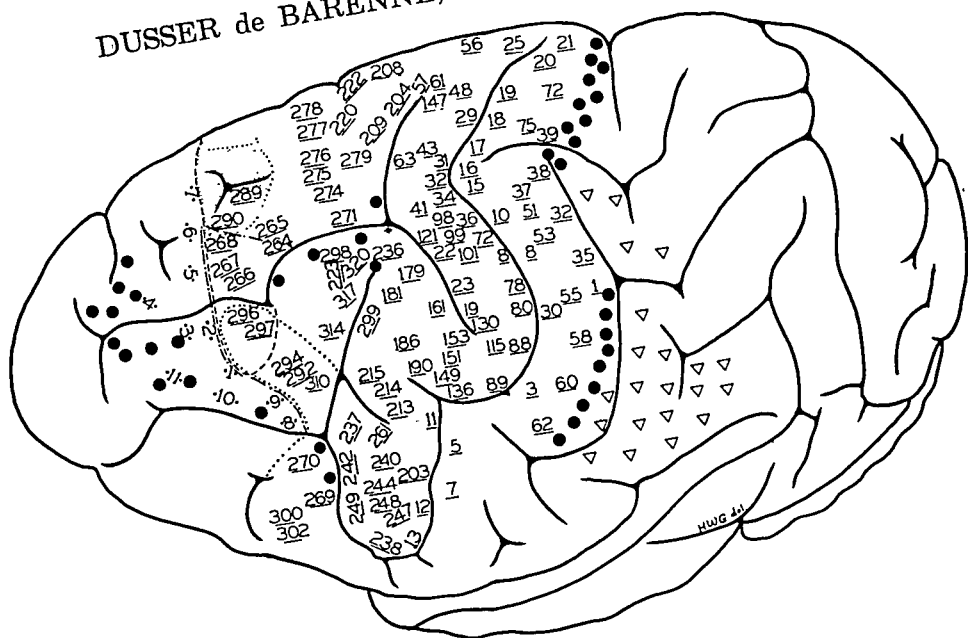


FIG. 4. Chimpanzee 11. May 28, 1940.

- Electrical stimulation here elicited
 - a) relaxation of existing muscular tension
 - b) suppression of motor response
 - c) holding in abeyance motor response only when there was existing
- ▽ Secondary facilitation elicited motor response of both fore and hind limbs, most marked on the contralateral side, but also some ipsilateral, accompanied by alternating forceful expirations and occasionally with suggested vocalization.
- The anterior boundary of the area eliciting motor response of the hind limb, upon facilitation.
- The anterior boundary of the area eliciting motor response of the fore limb, upon facilitation.
- The anterior boundary of the area eliciting motor response of the face, upon facilitation.
- * Motor point elicited contraction of the latissimus dorsi contralaterally. The numbered points indicate sites from which *primary responses* were obtained. They are divided into three groups: (a) precentral, (b) postcentral and (c) eye-field. The latter points are indicated by two dots adjacent to the numbers, corresponding to the exact site and separation of electrodes. The numbers of the other two groups are underlined, indicating the site of the electrodes, their distance apart being uniformly 3 mm.
 - a) Precentral motor points
 - V.D. 3000, f.p. 10 σ , 40 per sec., bipolar
 - 8 Flexion of elbow
 - 11 Lower lip to right
 - 12 Entire mouth to right
 - 13 Entire mouth to right
 - 19 Pronation of wrist
 - 22 Internal rotation of shoulder
 - 23 Flexion of elbow
 - V.D. 1500, f.p. 10 σ , 5 per sec., bipolar
 - 25 Plantar flexion
 - 29 Flexion of hip
 - 31 Adduction of thigh with slight flexion of hip
 - 32 Contraction right rectus abdominis
 - 34 Contraction in region of serratus anterior
 - 36 Adduction of shoulder
 - 41 Contraction of serratus anterior
 - 43 Adduction of thigh
 - 48 Plantar flexion

Description of Fig 4—Continued

- V D 1500 f p 10σ, 1 per sec, bipolar
56 Opposition of hallux
- V D 1500, f p 10σ, 50 per sec, bipolar
57 Flexion of all toes with inversion of foot
61 Flexion of toes with inversion of foot
63 Extension of knee
- V D 1500, f p 10σ, 1 per sec, bipolar
72 Flexion of elbow—mainly m brachialis
78 Flexion of elbow—mainly m biceps
80 Flexion of elbow with slight pronation
- V D 1500, f p 10σ, 50 per sec, bipolar
88 Flexion digits 2, 3, 4 and 5 of hand
89 Flexion digiti indicis only
- V D 1500, f p 10σ, 1 per sec, bipolar
98 Contraction of trapezius
99 Contraction of anterior half of m deltoideus
101 Flexion of elbow—m biceps alone
115 Adduction digiti minimi quinti of hand
121 Contraction in posterior axillary fold (latissimus dorsi)
130 Pronation at wrist (pronator teres and ? pronator quadratus)
136 Flexion digiti indicis only
147 Dorsiflexion foot
149 Flexion digiti indicis only
151 Flexion digiti minimi quinti only
153 Flexor carpi radialis
161 Slight contraction triceps
179 Contraction triceps only
181 Flexor carpi radialis
186 Flexor digitorum sublimis
190 Flexor digitorum profundus
203 Retraction corner of mouth
204 Plantar flexion
208 Plantar flexion
209 Plantar flexion
213 Elevation of lower lip
214 Drew down and protruded lower lip
215 Right orbicularis oculi with retraction of pinna
220 Dorsiflexion of foot
222 Plantar flexion
223 Flexion elbow (biceps)
- V D 2500 f p 20σ, 1 per sec bipolar
236 Right rhomboids only
237 Lower lip to right
238 Mouth to right
240 Mouth to right—chiefly lower lip
242 Both lips to right
244 Lower lip to right
- 247 Lower lip to right
248 Lower lip to right
249 Lower lip with slight upper lip to right
261 Mouth to right, chiefly lower lip
264 Flexion in flank with slight flexion of hip
265 Flexion of hip with slight flexion of flank
266 Flexion of hip
267 Flexion of knee
268 Flexion of knee
269 Lower lip to right
270 Lower lip to left with protrusion of lips
271 Latissimus dorsi—adduction and retraction of arm
274 Deep flexors of hip (iliopsoas?)
275 Flexion of knee
276 Dorsiflexion foot
277 Dorsiflexion foot
278 Plantar flexion foot
279 Plantar flexion foot
289 Flexion of pelvis hip and knee
290 Contraction in flank
292 Pronation of forearm
294 Flexion of fingers (flex dig pro fundus)
296 Supination (brachioradialis)
297 Flexion of fingers (flex dig pro fundus)
298 Flexion elbow (biceps with brachioradialis)
299 Flexion and pronation of wrist
Right orbicularis oculi only (contralateral blinking) obtained from here to just rostral of 237
300 Mouth to right
302 Mouth to right
310 Pronation of forearm later slight flexion of fingers
314 Pronation of forearm (pronator teres)
317 Slight flexion of forearm (brachialis)
320 Slight flexion of forearm
- b) Postcentral motor points
V D 2500, f p 20σ, 7 per sec, bipolar
1 Pronation forearm
3 Flexion of fingers (flex dig pro fundus)
5 Lower lip to right
7 Corner of lower lip drawn down
8 Extension of elbow internal rotation of shoulder
10 Latissimus dorsi—adduction of shoulder
15 Contraction of abdominal wall

Description of Fig. 4—Continued

16 Flexion at hip (iliopsoas?)	60 Flexion all digits of hand
17 Flexion of hip	62 Flexion of index finger only
18 Extension of knee	72 Dorsiflexion of foot
19 Plantar flexion of foot	75 Extension of knee
20 Opposition of hallux	c) Eyefields
21 Flexion of all toes	V.D. 1500, f.p. 10σ, 45 per sec., bipolar
30 Pronation forearm with slight flexion at elbow	1 Oculi contra and slight upward rotation
32 Internal rotation of shoulder with adduction of arm	2 Oculi contra only
35 Pronation forearm	3 Oculi contra only (same with V.D. 200)
37 Deep muscles of shoulder girdle	4 Same
38 Trunk muscles (erector spinae?)	5 Same
39 Flexion of hip	6 Same but less rapidly
51 Adduction of shoulder	7 Oculi contra but slowly
53 Flexion of elbow (biceps)	8 Oculi contra
55 Flexion of elbow (biceps) with pronation	9 Same
58 Flexion all digits of hand	10 Same
	11 Same

evidence summarized in the foregoing statements of the findings, we would cite a fair sample of the results of stimulation of the left hemisphere of chimpanzee 11, on which over 500 individual stimulations yielded discrete responses. These are given in Fig. 4 and its legend.

DISCUSSION

A survey of previous results obtained by electrical stimulation of the cortex in any species^{4,13,16-18,22,43-48,54-56,59,61,65,67,68,70-73,77,80} discloses great variation in findings, not only from observer to observer but from observation to observation by the same observer. The work on the chimpanzee is no exception.

When one seeks the reasons for the variation he finds these fall into one of three groups related either to the animal used, the conditions of the experiment or the type of stimulation.

Most, if not all, of the electrical stimulations reported on the chimpanzee^{8,13,54-56,61} have been on *Pan satyrus* but the age and physical status of the animals have varied widely. As the group reported here ranged from 2.5 to 3.5 years and were all in good nutritional state they were to this extent alike. The configurations of the hemispheres on the other hand were so variable, even between the two hemispheres of the same animal, as to render a comparison of detailed results difficult. Fortunately, the mapping of the functional organization of the sensory cortex, which followed each stimulatory investigation, permits one to be sure in which physiologically unique band a given stimulation was performed. Without this information no such general map as Fig. 5 could be justified. Even then, however, the difficulties of fitting the findings on any particular hemisphere into the schema representing all hemispheres (those in our collection as well as those described by Mingazzini,⁶⁶ Retzius,⁶⁹ Connolly²¹ and others) must be kept in mind.

Most of the electrical stimulations of the cortex of the chimpanzee have been done under ether or ether and chloroform anaesthesia. In the group re-

ported here ether was administered only long enough to render the animal manageable for administering Dial. Under its influence the threshold for electrical stimulation of the cortex remains low and relatively constant in contrast to the findings of Bucy^{16 17} and Bucy and Fulton.¹⁸ Moreover, operations performed under Dial are not attended by vasodilatation or a rise in blood pressure commonly encountered when ether is used. While there is no generally accepted method for measuring the depth of narcosis, it may be appropriate to state that, under Dial as employed here, the electrocorticogram

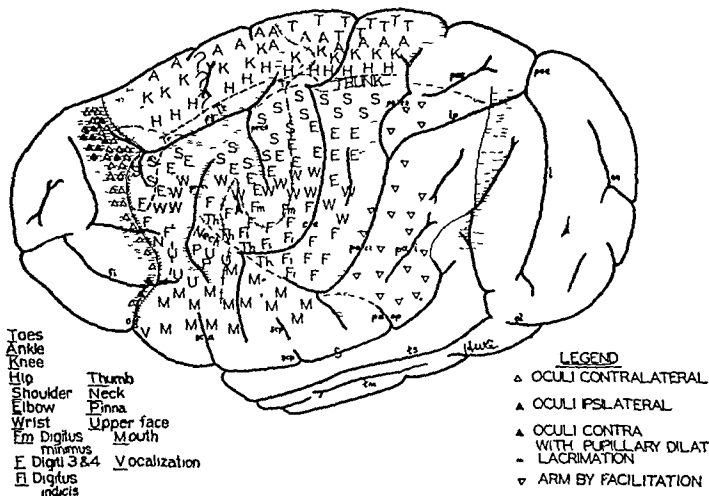


FIG 5

was essentially similar to the electroencephalogram found in man in normal sleep,²³ but the tension of the muscles was relatively high, holding the limbs in a persistent state of semiflexion, although the corneal reflexes were absent and no responses could be elicited by nocuous stimuli.

The exposure of the brain was made with extreme care, without trauma to the brain or to the dural vessels draining it except those which had to be thermocoagulated to permit a proper field of experimentation. In all cases enough vessels remained to ensure adequate circulation. The arachnoidal membrane over the cerebral hemisphere was not ruptured and the air of the room was kept around 80°C. and of high humidity, but the surface of the arachnoid membrane was not irrigated with saline solution as is done during operations on the human brain. The cerebrospinal fluid in the subarachnoid spaces was relied upon to keep the underlying cortex moist. Neither inspissation nor any unexpected threshold changes^{20 51} were encountered in the

first ten hours. Thereafter inspissation began, threshold rose and pledgets of warm Ringer's solution had to be applied at intervals in order to restore electrical excitability to about its previous level.

The fall in blood pressure under Dial⁵⁰ was compensated by elevating the foot of the animal board so that the heart was on the same level as the head.

Ag-AgCl electrodes, small spherules on the end of long pliable silver wires, were applied lightly to the cortex and that only during stimulation, thus minimizing local ischemic changes.

Type of stimulation—The first group of experiments reported here, namely those of facilitation and extinction, are sufficient to indicate how the responses obtained depend upon the parameters of stimulation. As has been indicated in previous studies on the macaque,³⁵ it is indeed easy to alter and even reverse the response¹³ elicited from a given focus by antecedent stimulation there or elsewhere, provided it be of the right kind and at the appropriate interval. This is, in fact, sufficient to account for such phenomena as deviation of response, reversal of response, etc. It was to prevent just these alterations induced by stimulation that the type of stimulation here employed was devised for mapping the cerebral cortex. Higher frequencies, longer pulses and greater duration of stimulation are too apt to induce extinction, which disappears only after a prolonged interval, thus delaying the experiment. The higher voltage required with shorter wave forms, shorter periods of stimulation and lower frequencies inevitably increases the spread of current, and thus induces relatively complicated or diffuse movements. If time were no object one would, of course, select approximately 60-cycle stimulation to obtain even more discrete results, whereas, if the object were to obtain the maximum number of responses in a given time, he would select very short, high voltage thyatron pulses and expect diffuse results, and even those from only a small fraction of the cortex which can yield motor response upon adequate stimulation.

While everything mentioned above doubtless plays some part in determining the differences in findings, probably the biggest factor responsible for the relatively small area which yielded motor responses to the earlier investigators was the unsuspected presence of suppressor bands.^{2,34} Even when their existence is known, until they have been delimited they remain most disturbing. For when one seeks the anterior or posterior margin of a region yielding "motor" response and, by chance, stimulates a band before or behind that region he induces a suppression of motor response which, unless the muscular tension be sufficiently great for relaxation to appear as a response, is manifest only as an inability to obtain a motor response from a point which previously yielded one to the same stimulus. The works of Leyton and Sherrington and of Graham Brown suggest that accidental stimulation or spread of current to Band III prevented them from obtaining responses from the more anterior portion of Bands IV and V, and, similarly, that accidental stimulation of Band VII prevented responses from Band VI, though this may have depended partly on the wave form which they used. It is

doubtful whether the experiments presented here would have been any more successful, had we not been forewarned by the results of our previous work on the suppression of motor response in the macaque.^{36,38}

That there was a significant spread of current in the experiments of Leyton and Sherrington is suggested from the size and shape of the motor eyefields, as shown in their diagram (Fig. 6). As the length of the stimulating impulse is increased and the voltage diminished, the eyefield becomes narrower.

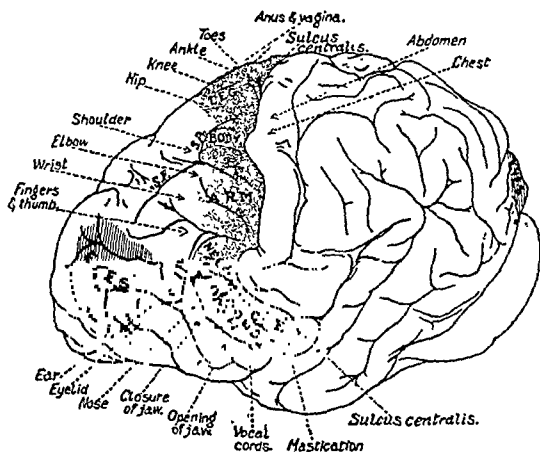


FIG. 6. From Grünbaum and Sherrington.

For example, when one traces point to point by "secondary facilitation" from the sulcus centralis, anteriorly or posteriorly, the suppressor bands interfere with the procedure, for if, in so doing, these bands are stimulated, no further responses are obtainable. One has, therefore, first to delimit these suppressor bands so that he can omit stimulation within them and resume it beyond them. A typical example for the anterior boundaries of leg, arm and face area is given in Fig. 4, as is also an indication of the far postcentral regions from which one can obtain motor responses by *secondary facilitation*, starting from foci in the postcentral gyrus which yield primary motor responses. It should be noted, however, that the response elicited from these far postcentral regions depends on the presence of muscular tension in the animal, a condition which is prevented by stimulation of Band VII or any other suppressor band. This is of especial interest for, while the nature and distribution of the factors for facilitation and extinction are fairly well known, neither the mode nor the site of operation of suppression of motor responses is yet established. Yet it would be as premature to attempt to con-

jecture what part this loss of tension may play in suppression of motor response as it would to attempt to explain how stimulation of the suppressor bands can hold in abeyance motor after-discharge following cortical stimulation. The outstanding fact which will have to be taken into consideration, in forming any hypothesis as to suppression of motor response, is this: it is obtainable from exactly the same areas as those which yield a suppression of electrical activity of the cerebral cortex.²

A comparison of the sensory cortex of the chimpanzee (Fig. 5) with that of the macaque³⁰ (see fig. 1) reveals an essential similarity of functional organization which not even the great discrepancy in size, shape and functional differentiation of the hemisphere can conceal.

It was hoped that in the work on the chimpanzee finer differentiations of function could be distinguished, as, indeed, was sometimes possible. However, the results with respect to Band I were in the main disappointing, for, while it was possible to find separate foci for a large number of discrete eye movements, in every hemisphere these occurred among foci for suppression, whether judged by relaxation of muscular tension, suppression of motor response or holding in abeyance an after-discharge.

Band II, lying immediately behind Band I, shows strikingly similar properties in both its "motor" and its "sensory" functions. It will be recalled that strychninization of any subdivision of this, the most anterior band of the sensory cortex, produced disturbances which were not confined to the subdivision of the cortex strychninized and in this same band, though primary movements restricted to some part of the extremity subserved by the subdivision in question could be elicited, experiments involving facilitation showed a wide overlapping seen nowhere else in the sensory cortex. The anterior boundary of Band II is fixed and easily found because of the suppression elicited from the band immediately in front of it. The same is true of its posterior boundary throughout the arm and part of the face regions, but in the leg region the posterior boundary is more difficult to locate, lying anterior to the ascending ramus of the superior precentral sulcus.

If one regards the superior precentral and the adjacent superior frontal sulci as comparable to the superior precentral in the macaque, and the ascending ramus of the inferior precentral as a homologue of the spur of the arcuate, Band III obviously represents area 4s, the "strip" in the macaque.⁵⁷ This should indicate a cytoarchitectonic division between these areas which Brodmann numbered 4 and 6 and which have been called respectively the "motor" and "premotor" regions.^{49,79}

In this case area 4 is divided into two bands, IV and V. They are dissimilar as regards both functional organization of the sensory cortex and threshold to electrical stimulation, a difference well known in the macaque (Hines). It is interesting in this connection to note that for cytoarchitectonic reasons von Economo^{40,41} divides Brodmann's⁵ area 4 into two bands, FA and FA_γ, which may correspond to our Bands IV and V in the chimpanzee.

The electrical excitability of the immediate postcentral band, VI, is

entirely in harmony with the findings reported in the literature concerning monkeys,^{27 43 72} orang,⁴ and man^{44-46 59} and enough has been said of the discrepant findings in the chimpanzee. Its posterior margin conforms curiously closely to that found in man. That margin is bounded by the suppressor region, Band VII, which has its functional homologue in the brain of the macaque (area 2s). While no previous investigators have reported responses to stimulation posterior thereto the conditions have never been the same.

That responses could be elicited by electrical stimulation of the posterior parietal sensory cortex, Bands VIII and IX, was, in view of statements to the contrary in the literature, most surprising. The only definite indication that movements could be elicited from this region in the monkey is given in the drawing of C. and O. Vogt^{78 (see p. 438)} and from this region in man cited by Foerster.⁴⁴⁻⁴⁶

The failure to obtain ocular movements from this same region seems, in view of the findings in the macaque, to indicate that the stimulus here employed did act upon the cells of this region rather than upon some subjacent fiber system and, therefore, tends to strengthen rather than to weaken the importance of our findings of motor response from this region.

The posterior margin of this "excitable" or "motor" posterior parietal region may well be defined by the suppressor band, XI, which lies just outside the sensory cortex.

This suppressor band, XI, which has its counterpart in the macaque³⁶ lies in a region whose position and shape suggests area 19 of Brodmann. With respect to results of electrical stimulation it resembles the three suppressor bands previously described.

The major conclusion of these investigations on the chimpanzee, covering both the functional organization of the sensory cortex and the "motor" response to electrical stimulation of the same hemispheres, is that "motor" responses can be elicited by appropriate electrical stimulation from practically all parts of the cerebral cortex which, by the criteria used, can be considered "sensory," except those bands, III and VII, where stimulation produced muscular relaxation instead of contraction.

Three considerations militate against the simple statement that this "sensory cortex" is also "the motor cortex." First, there is the factual difficulty with respect to the suppressor bands, III and VII, for while a relaxation is as truly a response as is a contraction, inclusion of bands giving relaxation would bring under this caption Bands I and XI, neither of which is "sensory" and one of which at least includes points motor for the eyes. Second, responses to electrical stimulation of the parietal regions depend, as shown in the experiments here cited, upon two adjuvant factors, namely, great tension in the muscles to be moved and facilitation of the secondary type beginning with the electrical stimulation of a point giving a primary response. The third consideration is a matter of terminology. For historical reasons the term "motor" cortex now means primarily "area 4," whereas the term "sensory" cortex has come to mean that region strychninization of which produces, in

the non-narcotized state, symptoms of somatic sensory excitation.^{24,25} Had the term "sensory" cortex been coined to designate that part of the cortex in which most discrete somatotopic localization is found,^{3,63} it would have had a much more restricted meaning, implying the postcentral region, par excellence. For all these reasons it seems best to state the conclusions as follows.

CONCLUSIONS

Appropriate electrical stimulation of the cerebral cortex of the chimpanzee under moderate Dial narcosis exhibits facilitation and extinction of motor response. Although their relation within a single response is more complex than in the macaque their dependence upon the parameters of stimulation is the same.

Stimulation with parameters selected to avoid facilitation and extinction, or to obtain facilitation when necessary to elicit responses from regions otherwise unresponsive, reveal the following:

With the exception of two narrow bands practically all parts of the cortex which, by the criterion here used, can be considered sensory yield contraction of skeletal muscles.

These two bands, III and VII, within (and two others, I and XI, just without) the sensory cortex yield (i) suppression of motor response to cortical stimulation, (ii) suppression of motor after-discharge following cortical stimulation and (iii) relaxation of skeletal muscles.

With the exception of the parietal area, which requires facilitation and existing muscular tension to exhibit motor response to cortical stimulation, the bands of the cortex revealed by electrical stimulation and motor response are identical with the bands revealed by strychninization and recording of electrical activity.

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FUNCTIONAL INTERDEPENDENCE OF SENSORY CORTEX AND THALAMUS*

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IN A RECENT paper on "Physiological delimitation of neurones in the central nervous system"⁹ the following statement was made: "This cortex showed, naturally, very little spontaneous activity due to the extirpation of its thalamus, by which procedure the cortico-thalamic-cortical circuits, necessary for the maintenance of the normal electrocorticogram, were destroyed." Substantiation would have been out of place there; we, therefore, present it here.

Monakow¹² was one of the first to recognize the existence of numerous corticothalamic neurones besides the well-known, sensory thalamocortical ones. In 1895¹³ he gave the first diagram showing the anatomical interrelation of the cerebral cortex and the thalamus. Subsequent experimental and clinical neuroanatomy have substantiated this finding (Kölliker, Déjérine, Flechsig, Bechterew, Minkowski, Poljak, Walker, Levin and others).

Head and Holmes¹¹ were the first to assign a function to the cortico-thalamic neurones, namely inhibition of the "essential centre" of the thalamus, i.e., of its medial nuclei which they presumed subserve the affectional side of sensation.

In previous papers the present authors^{3,4,8} have given the first experimental evidence of the functional interrelation of sensory cortex and thalamus, namely, that local strychninization of each of several of the constituent areas of the sensory cortex results in activation, "firing," of the sensory thalamic nuclei (as evidenced by the appearance of strychnine spikes in the electrothalamogram) and local strychninization of a sensory thalamic nucleus "fires" the corresponding subdivision of the sensory cortex.

Other experiments gave evidence that excitation of the sensory cortex excites the sensory thalamic nuclei^{1,2,6} and excitation of these nuclei excites the sensory cortex. In subsequent experiments it was established that local strychninization, or mechanical or electrical stimulation of a certain area of the sensorimotor cortex, area 4-s, results in a typical temporary suppression of electrical activity of other portions of the sensory cortex (area 4) and that this suppression is brought about via the caudate nucleus and the thalamus.⁸ Thus the operation of cortico-subcortico-cortical circuits was established. In this paper evidence will be presented that the "normal" electrical activities of the cortex and of the thalamus, even in the narcotized animal and without any especial excitation depend upon the intactness of their mutual connections, the cortico-thalamo-cortical circuits.

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† Deceased June 9, 1940.

METHODS

All experiments were performed on monkeys (*Macaca mulatta*) anaesthetized with Dial *. The electrical activity of the cerebral cortex was taken directly from its surface, i.e., the electrocorticogram (ECG) recorded, with bipolar electrodes feeding into a suitable amplifier and recorded with cathode ray oscillograph, Westinghouse oscillograph or inkwriter oscillograph (Grass). For simultaneous recording of the electrical activity of subcortical structures concentric needle-electrodes were used and their position checked at necropsy.

RESULTS

The problem is. What structures are necessary for the maintenance of the "spontaneous" electrical activity of the cerebral cortex, i.e., for the normal ECG, in the narcotized monkey?

It should be noted first that so long as the blood pressure or at least the blood supply of the brain can be maintained in the "decapitate" preparation, the electrical activity of the cortex appears unaltered. The term "decapitate" as here used means that the cord has been severed at the level of the foramen magnum. The same is found in the "decerebrate" preparation even when the level of section of the brain stem is above the superior colliculus. Figure 1 exemplifies these results.

Similarly, division of the corpus callosum can be accomplished without significant alteration of the spontaneous electrical activity of the cortex. The only difficulties are technical. In our most successful attempts, the calvarium was removed bilaterally and section was made by retracting the hemisphere opposite to the one recorded so that the slight trauma produced by retraction would not cause a diminution of activity of the area to be examined.

Even in such a preparation the electrical activity remains if a small area from which to record is left intact and the rest of the cortex of the same hemisphere is removed subpially, i.e., by suction, leaving the blood supply to the remaining structures. Figure 2 shows the electrical activity recorded from the arm area 4 before and after subpial resection of various other

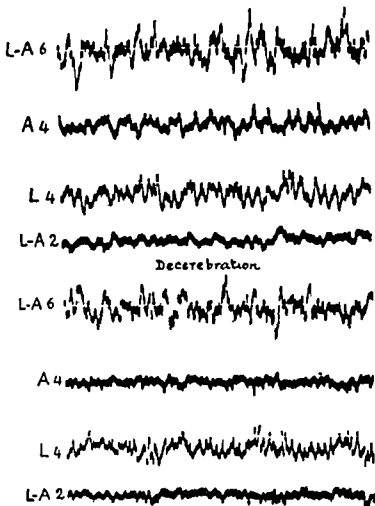


FIG 1 April 11, 1938 *Macaca mulatta*. Dial Curare Artificial respiration ECGs taken before and 1 min after decerebration

* The Dial was kindly put at our disposal by the Ciba Co.

cortical areas one after another until virtually all that remained on the convexity was the area recorded. This finding is confirmed by sectioning the cortico-cortical connections. Although the incisions pass all the way to the nucleus caudatus medially and the putamen laterally the activity is not significantly altered. Finally, gross lesions of the basal ganglia do not appreciably diminish the activity of the cortex. In fact, as has been stated else-

where, even small lesions of the nucleus caudatus are followed by augmentation of activity for a matter of hours. Here it should be stated that in no one animal have all of these lesions been made. However, so many combinations have been made that it is difficult to imagine any way in which this could lead to a false conclusion.

The results can, therefore, be summarized briefly. Essentially normal electrocorticograms can be obtained subsequent to any lesion or combination of lesions that does not compromise the cortical area whose activity is recorded, the thalamus or their interconnections.

When one asks not what may be destroyed and leave normal electrical activity in a cortical area, but what lesions prevent it, the following results are significant.

Obviously the entire cortex cannot be destroyed and an ECG obtained. In previous publications from this laboratory^{5,7,14} it was shown that laminar thermocoagulation of all layers external to that of the large and giant pyramidal

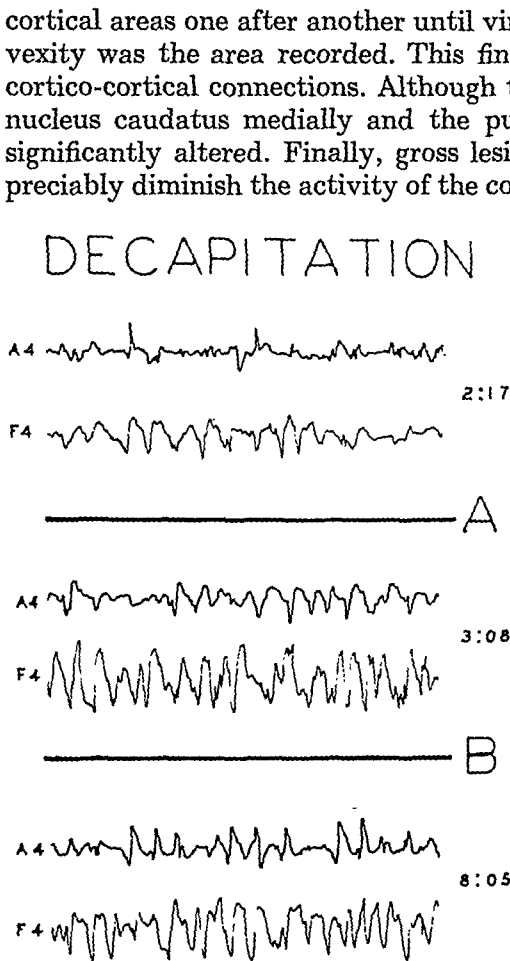


FIG. 2. Nov. 17, 1939. *Macaca mulatta*. Dial. Decapitation at 11:22.

15 mm. per sec. 6.5 mm. = 20 μ V.

A = Removal of postcentral sensory arm cortex by suction at 2:23.

B = Stepwise removal of all sensorimotor cortex except A4 and F4 (3:15 to 7:45). (Reduced to $\frac{2}{3}$ size)

cells while temporarily obliterating almost all activity did not produce any permanent deficit or alteration, whereas thermocoagulations which included the layer of large and giant pyramidal cells and left only the polymorphic layer produced a permanent and profound reduction of electrical activity.

If one makes a lesion in the ventro-lateral nuclei of the thalamus which have been shown to be sensory¹⁰ and which interrelate with the sensory cortex as mentioned above, then the corresponding subdivision of the sensory cortex shows a diminution of its electrical activity. Here it is important to

note the lack of change in the ECG of other areas, Figure 3 exemplifies both findings.

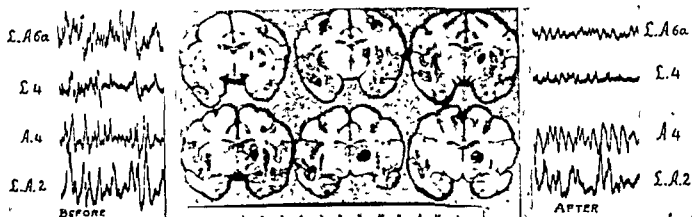


FIG. 3. March 18, 1938. *Macaca mulatta*. Dial. ECGs taken of right hemisphere before and 1 hour and 40 min. after lesion of leg-nuclei of right thalamus.

Next, if one severs the connections of the cortex and thalamus, by deep undercutting, the cortical activity immediately disappears. This is seen in Fig. 4.

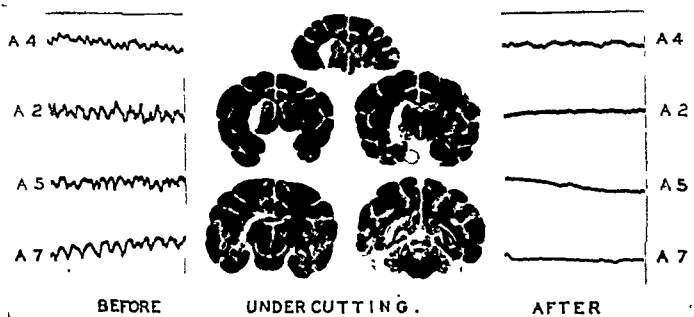


FIG. 4. Feb. 1, 1937. *Macaca mulatta*. Dial. ECGs taken before and after deep undercutting of right sensori-motor cortex.

It should be added that such deep undercutting leaves the blood supply of the cortex so good that its threshold to electrical stimulation, judged by the appearance of electrical after-discharge, is unaltered. One other result of such a lesion is of interest here, namely, that the electrothalamogram (ETG), in spite of the intact afferent tracts from lower levels, shows an almost equal reduction.

No animal with such an extensive undercutting has been kept for a

sufficient length of time to determine how much activity might return. With a small lesion cortico-cortical connections might well be expected to play an important role in restoration of activity. Even in the acute experiment this supposition finds support, for the ECG of an area locally undercut is never completely inactive.

Nevertheless it was considered worthwhile to know what permanent alterations would follow from a relatively small undercutting of the sensory cortex. A lesion was therefore made under the medial half of arm area 4 and extending partly under leg area 4. This animal was kept for over 2½ years to allow complete retrograde degeneration to obliterate all cells whose axones

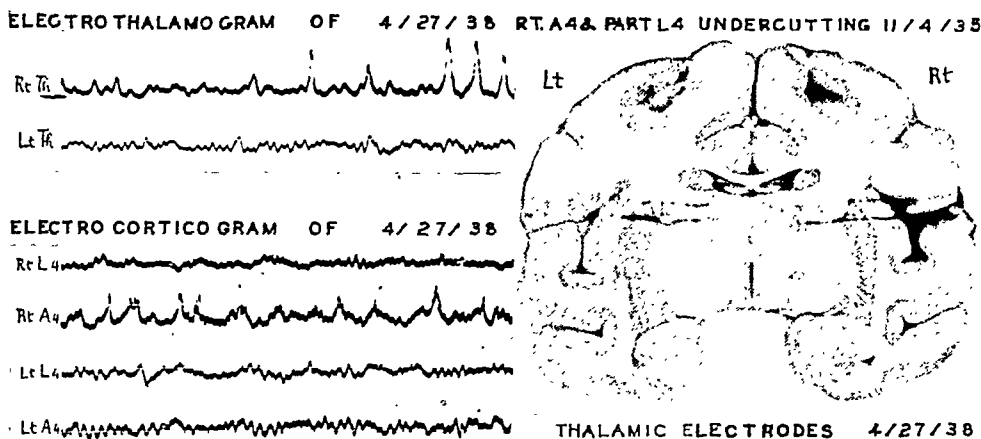


FIG. 5. April 27, 1938. *Macaca mulatta*. Dial. Last ECGs taken 2.5 years after undercutting of A4 and part of L4 of right hemisphere. Animal at rest with its eyes closed showing alpha frequency circa 10 per sec. in normal structures.

had been severed. When the skull was opened the dura was not adherent over the areas to be studied. In order to be certain that the findings were not unduly influenced by anaesthesia the cortical records were first obtained under light anaesthesia with the animal's head securely clamped. Thalamic electrodes, with shields grounded, were then inserted and records were made from them. The monkey was allowed to come out from under anaesthesia gradually and continued to lie quietly on the table. The records then obtained show an alpha rhythm from normal cortex and thalamus but abnormal activity from the cortex and thalamus which had been separated by undercutting 2½ years before. Figure 5 shows the location of the thalamic electrodes, and the last records obtained from this monkey resting undisturbed with his eyes closed.

DISCUSSION

Inasmuch as small lesions involving the deeper layers of the sensory cortex or the sensory nuclei of the thalamus or the tract through which these

are mutually related cause permanent local abnormality and loss in spontaneous electrical activity of the corresponding parts of both cortex and thalamus, whereas no lesion or combination of lesions elsewhere does so it seems appropriate to regard these, and only these, structures as essential to that activity. Since excitation of either of these gray masses has already been shown to excite the other and neither can support "spontaneous" activity in isolation from the other, it is clear that the activity of either depends upon activity reaching it from the other. That is, for spontaneous activity, the sensory cortex and the optic thalamus are functionally interdependent.

SUMMARY

When fall of blood pressure or other impairment of circulation is avoided, decapitation, decerebration, section of the corpus callosum, and subpial resection of the entire cortex of the convexity of the hemisphere, save that area to which electrodes are applied, one and all leave the electrical activity unaltered. So also does destruction of sensory thalamic nuclei other than that corresponding to the subdivision of the sensory cortex whose activity is being recorded. Lesions of the basal ganglia have little or no effect on the activity of the sensory cortex or of the corresponding thalamic nuclei, except for a transient increase following injury to the nucleus caudatus.

On the other hand a lesion of the sensory thalamic nucleus corresponding to a cortical area under investigation and a lesion of the cortical area corresponding to a sensory thalamic nucleus under investigation, each results in a permanent abnormality and diminution of spontaneous electrical activity on the other. The same obtains when their mutual connections are interrupted.

Laminar thermocoagulation of the superficial layers has previously shown that the layers of the cortex external to that of the large and giant pyramidal cells are not necessary to the maintenance of the normal ECG.

It is therefore concluded that, for the maintenance of the normal spontaneous electrical activity of the sensory cortex and of the corresponding sensory thalamic nuclei, the essential structures are the deeper layers of the cortex, the corresponding thalamic nuclei and their mutual connections, and, hence, that for this activity the sensory cortex and the ventro-lateral thalamic nuclei are functionally interdependent.

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SUPPRESSION OF MOTOR RESPONSE OBTAINED FROM AREA 4 BY STIMULATION OF AREA 4s*

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IN 1934 during the investigation of facilitation and extinction of motor response to cortical stimulation^{1 2 3 6 7 8 9 12 17 18 19} due to antecedent stimulation of neighboring foci a new phenomenon appeared when the antecedent stimulation was in either the frontal part of area 4 or in the posterior part of area 6. When stimulation of this region preceded by several minutes the testing stimulation of area 4 no response to that stimulation of area 4 was obtained. Careful examination of the records showed frequently a slight lowering of the base line, suggesting a relaxation of the extremity. During mapping of the functional organization of the sensory cortex,¹⁰ the region from which the new phenomenon could be elicited was discovered to give, upon local strychninization within it, a diminution of the electrical activity of area 4. The area yielding these phenomena, "suppression of motor response" and "suppression of electrical activity," was designated area 4s and was found to coincide with the strip of cortex from which Dr. Marion Hines obtained, by electrical stimulation, a cessation of movements and a relaxation of contracted muscles¹⁴ and ablation of which by the same author produced spasticity.¹³

In a previous publication on the functional organization of the sensory cortex¹⁰ it was shown that the "firing" of one area by another was dependent upon cortico-cortical connections which remained intact when the cortex was severed from deeper structures, by deep undercutting, whereas, in a subsequent publication,¹¹ it was demonstrated that the suppression of electrical activity of area 4 by strychninization of area 4s was dependent upon a one-way circuit from area 4s to the nucleus caudatus, thence to the thalamus and so to the cortex. As it was hoped, at that time, that the analogous phenomenon, the suppression of motor response, might readily be traced either in the cortico-cortical system or, more probably, through the corresponding deeper structures the publication of a full description of the phenomenon was delayed until the experiments could be performed to demonstrate its circuit. This proved more difficult than was expected, wherefore it was deemed wisest to write the present article in which the phenomenon is fully described, the distinction between it and the extinction of motor response made clear and, finally, the attempts to trace its path through the central nervous system are recorded together with the reason for each experiment.

* Aided by a grant from the Fluid Research Funds of the Yale School of Medicine
† Deceased June 9, 1940.

METHODS

All experiments were performed upon monkeys (*Macaca mulatta*) anaesthetized with Dial* (0.45 cc. per kg. body weight, $\frac{1}{2}$ the dose intraperitoneal, $\frac{1}{2}$ intramuscular). The cortex was exposed and stimulated with thyatron impulses delivered through Ag-AgCl electrodes. Responses were recorded isotonicly on a smoked paper kymograph. When necessary these procedures were supplemented by recording the electrical activity of various structures of the central nervous system. This was done with moving paper camera and 4-channel Westinghouse oscillograph with appropriate amplifiers.

Lesions were made chiefly by a small blade mounted on a mechanically controlled needle which could be thrust into the brain at any required angle to any required depth, there rotated and then returned to the original position before retraction, thus minimizing the lesion of approach.

Brains with lesions were taken up into a sarcophagus with agar (of approximately the same consistency as the brain), extruded against an angular mount, sectioned with a spatula into slices either 1 or 2 mm.,⁴ and photographed.

RESULTS

Stimulation of the cerebral cortex with a manually applied electrode by well controlled thyatron impulses discloses that the threshold rises fairly

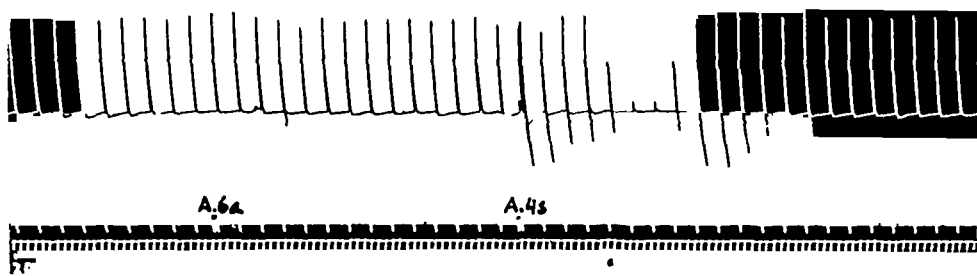


FIG. 1. Sept. 27, 1938. *Macaca mulatta*. Dial. Electrical monopolar stimulation (Thyatron) of A4 focus once every minute (5 sec.-0.6 μ F.-40 per sec.-V.D. 1200). Extension of wrist. Electrical stimulation of A6a focus gives no suppression. Electrical stimulation of A4s focus (6 sec.-1 μ F.-40 per sec.-V.D. 7000) gives suppression. Time line = 20 sec.

abruptly as one passes from the posterior to the more anterior part of area 4. This change is encountered in about the middle of area 4 and is of the order of a 100 per cent rise in threshold. If the impulses are relatively long this threshold is not very different from that of area 6, but between area 4 and area 6 lies a narrow band of cortex (2 or 3 mm. wide and running dorso-ventrally through the anterior part of the superior precentral sulcus) where stimulation fails to elicit any contractions of muscles until such voltages are reached that spread of current must have involved the adjacent areas. This band of cortex from which no contraction of muscles is elicited is the strip of Marion Hines,^{13,14} area 4s, i.e., the strip from which one obtains a suppression of electrical activity of area 4. But the stimulation of area 4s, although it fails to elicit contraction, has (as Marion Hines has shown) definite effects which indicate that it has excited the cortex.

* We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

Figure 1 shows the effect with which this paper deals, namely, the suppression of motor response to electrical stimulation of a motor focus of area 4 by antecedent stimulation of area 4s. If one stimulates a focus of area 4 once a minute with such a stimulus that neither facilitation nor extinction disturbs the amplitude of the successive responses and then one stimulates area 4s the responses to the area 4 stimulation diminish or disappear.

Figure 2 shows that this suppression of motor response can be brought about by chemical or mechanical as well as by electrical stimulation of area 4s, and therefore cannot be due to inadvertent stimulation by spread of current to area 4 or to sub-jacent fibre tracts—a conclusion confirmed by thermocoagulation of the entire thickness of the cortex which prevents suppression.

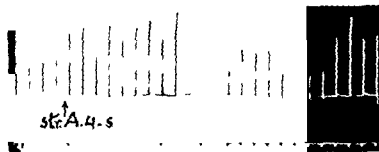


FIG. 2. April 13, 1939. *Macaca mulatta*. Dial. Electrical stimulation once every minute of focus of A4 for extension of fingers (5 sec.-0.5 μ F.-40 per sec.-V.D. 3600). Strychninization of A4s. Note 9 min. latency, and bimodal suppression.

Several characteristics of this suppression of motor response are noteworthy: (i) The suppression has a remarkable latency after electrical stimulation. The duration of latency most frequently encountered is 4 min., but latencies as short as 2 min. or as long as 12 min. have been obtainable by use of light anesthesia and strong stimulation of 4s for the shorter, and deep anesthesia and weak stimulation of 4s for the longer latencies. After mechanical stimulation the latency is usually short, whereas after strychninization, measured from the moment of application, the latencies are usually from 12 to 20 min. (ii) The suppression lasts several minutes. Suppression of motor response to 6 or 7 stimulations at one-minute intervals is ordinarily encountered. With weak stimulation of area 4s the duration is usually shorter. Suppressions lasting 20 to 30 min. are not uncommon with strong stimulation of area 4s in deeply narcotized animals. (iii) The suppression, if long, is frequently bimodal. Longer suppressions are occasionally trimodal. (Cf. Fig. 2, 5 and 7.) (iv) The suppression is accompanied by a change in muscle tension. During the suppression, sometimes starting before noticeable change in amplitude and sometimes lasting after amplitude has returned to its original level, there is a slight fall in the base line provided there is tension in the direction of gravity in the thread to the tambour in the resting position, thus indicating a loss of tension of the muscles supporting the extremity. Moreover, the last responses before, and several after the diminution (or absence) of amplitude look atonic and frequently graph the relaxed condition of the muscles by falling past the resting position. (Cf. Fig. 1 and 2.) (v) The suppression cannot be reinduced immediately. As these experiments were tedious attempts were made to find out how soon it was advisable to attempt to repeat the experiment. After a prompt, short suppression induced by me-

chanical stimulation of 4s, 15 to 20 min. suffices but with a typical 4-minute latency, 6-minute suppression following electrical stimulation of 4s, the experiment usually fails if attempted within 35 min. and succeeds at the end of 45 min.

The second group of findings are those that separate the suppression of motor response from the extinction of motor response to which it bears only a superficial resemblance.

The first, and obvious, difference is that the only part of the so-called motor cortex from which the suppression of motor response to cortical stimulation of any motor focus of area 4 can be obtained is area 4s; whereas extinction is obtained maximally by antecedent stimulation of one and the same focus and can be obtained

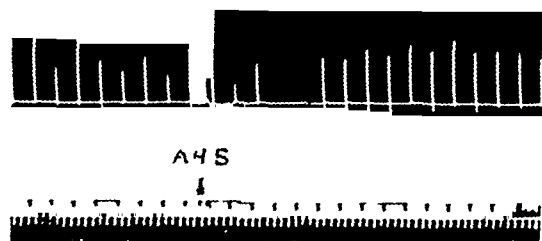


FIG. 3. Oct. 4, 1938. *Macaca mulatta*. Dial. Flexion of fingers. Stimulation of A4s (5 sec.-1 μ F.-50 per sec.-V.D. 6600) at posterior margin of A4s. Note small primary response. The responses to the following A4 stimulation (5 sec.-0.5 μ F.-50 per sec.-V.D. 3600) show (i), facilitation, (ii), extinction, (iii), return, then suppression.

by antecedent stimulation elsewhere only if the disturbance (notably, in the form of an after-discharge) actually involves the structures excited by the test stimulation. Whether such is the case when 4s stimulation produces unresponsiveness to stimulation of area 4 can be examined by electrical recording of the activity of the focus of area 4 to which the test stimulus is to be applied. The experiment proves that suppression of motor response can be elicited with no

trace of after-discharge in any part of the cortex.

The second difference between these phenomena is also essentially spatial. Except where antecedent stimulation has been so extreme as to initiate an after-discharge so violent as to spread across the functional boundaries between the face-, arm- and leg-subdivisions, extinction has always remained localized to the subdivision stimulated; whereas the suppression of motor response to stimulation of a motor focus of, let us say, A4 can be elicited by antecedent stimulation of L4s, A4s, F4s on the same hemisphere and even, although less markedly, by stimulation of 4s on the opposite hemisphere.

The third difference is temporal. Except for the belated components of extinction due to the slow progress of a persistent after-discharge, which have been carefully excluded, extinction lasts a matter of one or two minutes at the most, whereas suppression has a latency of some four minutes. To make perfectly certain of the temporal separation in a single suppression one has only to place the 4s electrodes on the posterior margin of area 4s of the same subdivision as that of the area 4 focus used in testing and to increase the voltage of the area 4s stimulus sufficiently so that spread of current stimulates the more anterior part of area 4. One obtains then, by proper

timing, facilitation to the first test stimulus, extinction to the second, no change to the third and suppression to the subsequent stimulations for the next several minutes. Figure 3 demonstrates this finding.

It is appropriate here to record another finding. At the suggestion of John Hamilton of this laboratory that the suppression of motor response from what was intended for stimulation of area 4s might be merely the effect of simultaneous stimulation of area 4 and area 6, two monopolar electrodes were placed one in area 6, immediately anterior to 4s, and the other in area 4, immediately posterior to area 4s and these were connected to the thyatron used for 4s stimulation. These monopolar electrodes were scarcely 3.5 mm apart. Yet, even with slightly higher values of stimulation than sufficed in area 4s no suppression of motor response occurred. Instead, there was a primary response to the conjoined stimulation, followed by the facilitation and subsequent extinction of response to area 4 stimulation.

Inasmuch as the suppression of motor response was not restricted either to responses elicited from one part of the cortex, or to responses of a single

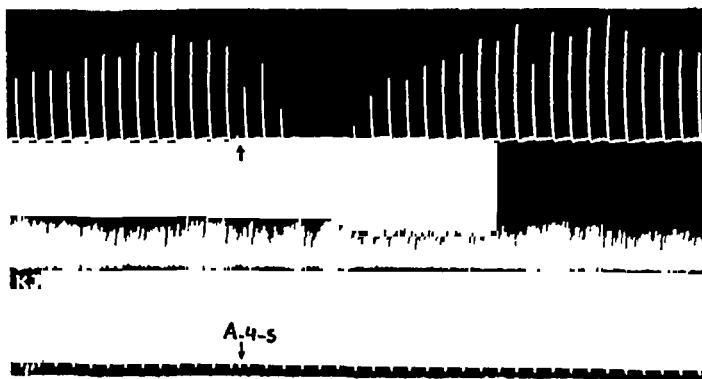


FIG 4 March 7, 1939 *Macaca mulatta* Dial A4 = 5 sec - 0.5 μ F - 50 per sec - V D 800 A4s = 5 sec - 1 μ F - 50 per sec V D 1600 Time line = 1 min Upper record, extension of wrist in response to stimulation of A4 focus Lower record, K J unaffected during suppression of motor response to cortical stimulation

part of the body, the question at once arose as to whether it might not be due to an induced systemic alteration of circulation, respiration or metabolism which, in turn, produced a completely generalized rise in threshold either throughout the central nervous system or throughout the musculature. To settle this question it seemed simplest to examine a response which was initiated by other than cortical stimulation. The knee jerk in response to weak mechanical stimulation of the patella tendon every 4 sec. was, therefore, recorded before, during and after a suppression of motor response. Figure 4 shows the result of this experiment. The knee jerk is not altered.

Thus one has to look for a disturbance in restricted parts of the central

nervous system and to attempt to trace its course from the area stimulated—area 4s—to the structures involved in the response to the testing stimulation of area 4. The simplest possible path which might be involved is a cortico-cortical connection from area 4s to area 4. However, section by a deep incision down to the nucleus caudatus does not prevent suppression. Figure 5 shows the suppression elicited after such a section.

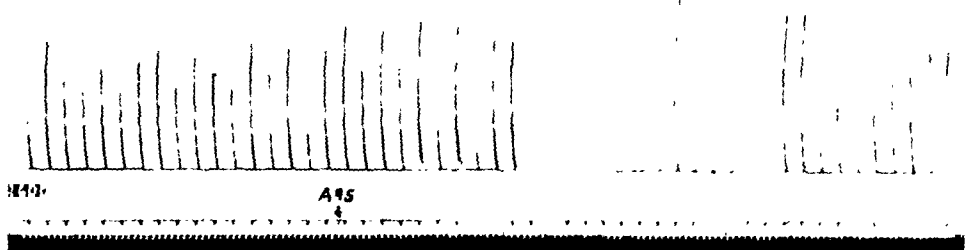


FIG. 5. Oct. 10, 1938. *Macaca mulatta*. Dial. Suppression of motor response at 9:05 P.M. (A4s=5 sec.-1 μ F.-40 per sec.-V.D. 5600. A4=5 sec.-0.5 μ F.-40 per sec.-V.D. 2600) subsequent to large lesion of nucleus caudatus at 5:00 P.M. and incision 13 mm. deep between A4s and A4 at 7:40 P.M.

Since one had to look, therefore, into cortico-subcortical connections, and since among these the cortico-caudate projection was known to be necessary for the suppression of electrical activity of area 4 upon strychninization of area 4s, an attempt was made to pith the nucleus caudatus by a frontal approach. This lesion prevented the suppression, but at autopsy, although the nucleus caudatus was extensively injured, the blade had been at such an angle as to sever the descending fibres from area 4s, and no conclusion could be drawn as to which part of the lesion was significant in the prevention of suppression.

Therefore, a small undercutting of 4s was made. This simple lesion prevented suppression and demonstrated that the disturbance necessary for suppression required a descending track. This is the direct proof of what one could never legitimately infer by exclusion from the results of severance of cortico-cortical connections.

Next a lesion was made in the nucleus caudatus without injury to the cortico-caudate fibres external to the caudate, by approaching it at another angle. The suppression of motor response was obtained again. Figure 6 shows the lesion and the suppression. This lesion is rather small but was sufficient to prevent any suppression of electrical activity of area 4 upon strychninization of area 4s even after the suppression of motor response had again been elicited after the lesion. Larger lesions of the nucleus caudatus also failed to prevent the suppression of motor response. One is therefore forced to conclude that the nucleus caudatus which is necessary for the suppression of electrical activity is either not necessary for the analogous suppression of motor response or that remaining parts of the nucleus caudatus, although

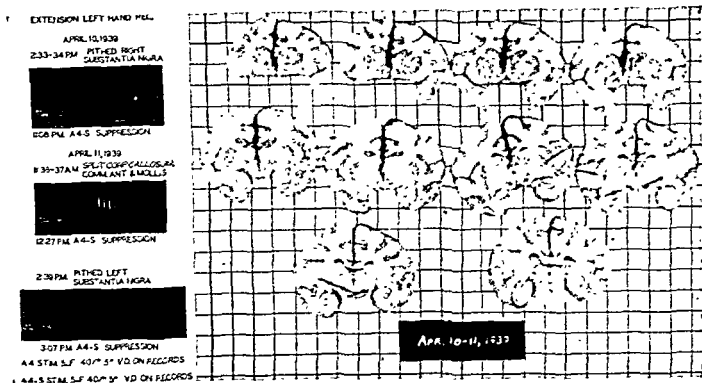


FIG. 8. *Macaca mulatta*. Dial. Suppression of motor response after bilateral lesions of substantia nigra and splitting of brain. For parameters of stimulation see records.

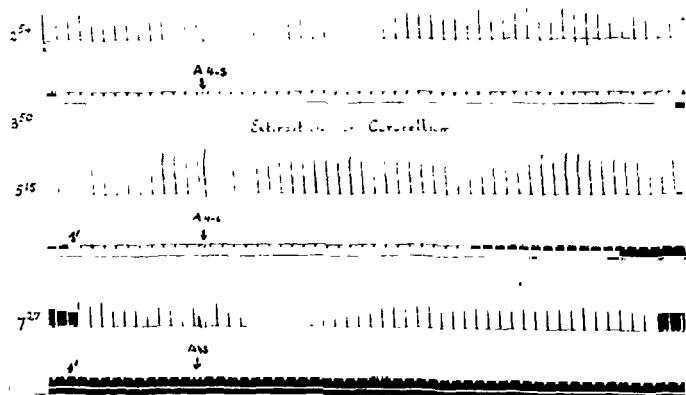


FIG. 9. Feb. 22, 1939. *Macaca mulatta*. Dial. Record 1. Suppression of motor response elicited at 3:50 ($A_4=5$ sec.-0.5 μ F.-50 per sec.-V.D. 2700. $A_4s=7.5$ sec.-1 μ F.-50 per sec.-V.D. 5400). Record 2. Poor suppression elicited shortly after total extirpation of cerebellum at 5:15 when threshold was high ($A_4=5$ sec.-0.5 μ F.-50 per sec.-V.D. 4100. $A_4s=7.5$ sec.-1 μ F.-50 per sec.-V.D. 8200). Record 3. Good suppression at 7:27 when threshold had returned to original value ($A_4=5$ sec.-0.5 μ F.-50 per sec.-V.D. 2800. $A_4s=7.5$ sec.-1 μ F.-50 per sec.-V.D. 5600).

in question. The reason for not reporting the negative cases even when they outnumbered the positive cases, is that one can deduce anything only from a positive finding. Failure to find suppression subsequent to a lesion may be due to any number of causes, but the presence of suppression after a lesion destroying some structure clearly indicates that that structure is not necessary for suppression. On looking over early records of such failures to obtain suppression it became clear that these failures usually occurred when, following the lesion, the threshold to electrical stimulation of area 4 has been raised and before it has returned to normal. Therefore, in later experiments, the suppression of motor response was sought about every hour until the

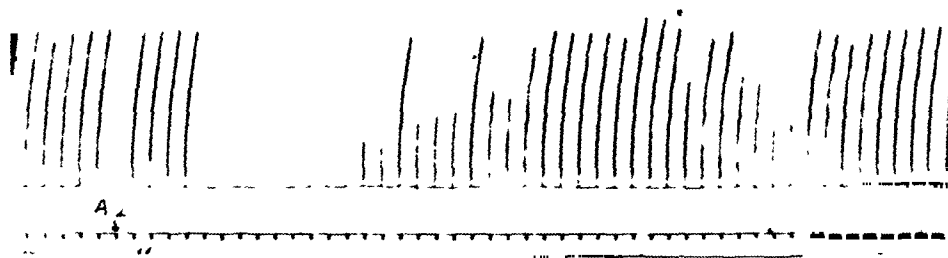


FIG. 10. April 19, 1939. *Macaca mulatta*. Dial. Suppression of motor response to electrical stimulation ($0.5 \mu\text{F}$.-50 per sec.-V.D. 1300) of A4 produced by stimulation ($1 \mu\text{F}$.-50 per sec.-V.D. 2600) of A2. Time line = 1 min.

threshold had been returned to its original value for at least one hour. This greatly reduced the number of animals that would have been required otherwise and proved that the return to approximately pre-lesion threshold was essential for suppression. (Cf. legend of Fig. 9.)

After these experiments had been completed it was discovered that stimulation in the postcentral gyrus also produced a suppression of motor response, and still more recently the same has been encountered from area 8 and from a strip of cortex lying immediately occipital to the sensory cortex. Figure 10 shows the suppression of motor response to electrical stimulation of area 4 by antecedent stimulation of the postcentral gyrus.

DISCUSSION

The suppression of motor response to stimulation of area 4 by antecedent stimulation of area 4s has obviously raised many more questions than the investigation has been able to answer. It is well, therefore, to consider first what the experimental facts prove even if these conclusions are negative. The phenomenon itself remains interesting and can always be elicited.

First, the phenomenon has been clearly demonstrated to be something other than extinction, for the two can be separated both spatially and temporally. Second, it cannot be explained as a result of the extinction of activity in area 4 due to stimulation of 4s, for suppression of motor response occurs after a lesion of the nucleus caudatus which prevents suppression of electrical

activity of area 4. Third, its long latency indicates an indirect, or complex, "delay" path. Fourth, its long duration, indicating a continuous activity of some sort (comparable to that found for after-discharge following strong cortical stimulation), together with the even longer subsequent period during which the suppression cannot be reinitiated (comparable to the duration of extinction following such an after-discharge) indicate that one is probably dealing with an activity antagonistically related to that of the motor system excited by stimulation of area 4 rather than with extinction of activity contributory to that motor system. If these suppositions be correct it follows that one is here dealing with augmented activity in a system antagonistic to the cortico-spinal system, and it becomes proper to regard suppression of motor response as an example of "inhibition," although not in the sense in which Sherrington originally used the term to designate the relaxation of the antagonist during contraction of the reciprocally innervated agonist, since, in suppression, the relaxation involves all muscles observed. The antagonism is not between agonist and antagonist muscles but between pyramidal and extrapyramidal systems within the central nervous system and there is no evidence that activity of area 4 is antagonistic to activity of area 4s. If the antagonism is not mutual there is no reason to regard the relation between the pyramidal and extrapyramidal systems as reciprocal.

One more observation tending to the same conclusion may be cited here. In the paper concerning the motor cortex of the chimpanzee⁵ it is stated that the motor after-discharge (following stimulation of areas giving motor response) was held in abeyance during stimulation of suppressor bands which have been shown to correspond to 8s, 4s, 2s and 19s in the monkey. While this finding was obtained by use of more appropriate stimulation (*i.e.*, of longer pulse form) than was used in the experiment reported here and the work was on another species, it still seems significant to mention it here because it was obtained from the bands yielding suppression of motor response as here described, and suppression of electrical activity. Another previous finding, namely, that the deeply undercut cortex could support a prolonged after-discharge, indicating the essentially cortical nature of this type of after-discharge, shows that the holding in abeyance of the motor after-discharge can only be interpreted to mean that cortico-spinal impulses continued to be delivered to the cord but that its motor horn cells were rendered unresponsive by impulses traversing other descending systems. In this case there is no possibility of extinction; *per contra*, as the stimulus is there at the time and is adequate, there is every reason to expect that some system whose activity is antagonistic to cortico-spinal activity is contemporaneously active.

Thus consideration of time relations, of co-present relaxations and of holding in abeyance of after-discharge, all indicate activity in a system antagonistically related to the cortico-spinal system, *i.e.*, "inhibition" of the response to cortico-spinal activity by contemporaneous activity of some antagonistic extrapyramidal system.

The chief difficulty with this hypothesis arises from that constancy of the knee jerk in response to weak patellar stimulation which proved that the path of the "inhibiting" disturbance was within the central nervous system. It is hard to see how the motor horn cells involved could respond normally to dorsal root stimulation and not to cortico-spinal stimulation. The difficulty may be due to insufficient knowledge of the events in the spinal cord, but until these are known the notion that suppression of motor response is a differential "inhibition" of the final common path must be held reservedly. Alternative conceptions of the site of the hypothetical "inhibition" are confronted by anatomical objections.¹⁵

With respect to what structures of the extrapyramidal system are involved in this suppression the seemingly most "unsuccessful" experiments are most conclusive. They have shown of one structure after another that it is not necessary to this suppression, *i.e.*, that the remaining structures are sufficient. The only structures whose destruction has yielded a seeming "success" remain of questionable importance, namely, the ansa lenticularis, the fields of Forel and the red nucleus, and inasmuch as after the lesion was made the threshold of area 4 never returned to its original value these experiments are equivocal. What remains most surprising is that suppression of motor response does not depend upon an intact nucleus caudatus, for this has been shown to be the only part of the corpus striatum to which areas 8s and 4s send axones. Here the possibility remains that remaining parts of this structure sufficed for this suppression but not for that of electrical activity.

SUMMARY

Motor response to electrical stimulation of area 4 is suppressed (typically, after a latency of several minutes for a duration of 5 or more minutes) by electrical or other stimulation of area 4s. It cannot be initiated again for many minutes.

This suppression is brought about by stimulation of cells in area 4s and does not require stimulation of underlying fibre tracts.

It has been shown to be distinct from extinction of motor response by temporal and spatial consideration.

It depends for its occurrence upon fibre tracts descending from 4s to deeper structures and not upon cortico-cortical connections.

It is, despite its great generality, restricted in its distribution within the central nervous system, as is proved by its failure to affect the knee-jerk.

The following structures have been shown to be severally unnecessary for its occurrence: nucleus caudatus, putamen, globus pallidus, thalamus, substantia nigra and cerebellum.

It is at present best conceived as brought about by increased activity in some portion or portions of the extrapyramidal system whose activity is antagonistically related to that of the cortico-spinal system.

Finally, it can be obtained from areas 8s, 4s, 2s and 19s, *i.e.*, from all of those cortical areas from which suppression of electrical activity of the cortex

can be obtained, although its occurrence is not dependent upon the suppression of that activity.

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FUNCTIONAL ORGANIZATION OF SENSORY AND ADJACENT CORTEX OF THE MONKEY*

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IN PREVIOUS papers concerning the functional organization of the sensory cortex^{7,11} the boundaries of that area have been mentioned and it has been stated that strychninization immediately outside of it failed to fire any part of it. So clear was the evidence to this effect in *Macaca mulatta* that it was used as a criterion of the sensory cortex in the researches on the chimpanzee.^{2,3,4,10} The present study deals with the properties and relations of these adjacent regions and with detailed observations on constituent areas of the sensory cortex which had to be precisely determined in order to be certain as to the site of electrodes and strychninizations here employed. These experiments were interspersed among studies of the same regions on the chimpanzee's brain in which it was often possible to keep strychninization confined well within a single physiologically unique band and thus to obtain a clearer conception of what to attempt in the monkey. More precise knowledge of several areas has thus been brought to light so that, aided by occasional histological control, it is now possible to improve on the previous diagram of functional organization within the sensory cortex and to be relatively certain about cytoarchitectonic correlations.

METHODS

All experiments were performed upon monkeys (*Macaca mulatta* and one mangabey) fully anaesthetized with Dial§ (0.45 cc. per kg. body weight, $\frac{1}{2}$ the dose given intraperitoneally, $\frac{1}{2}$ intramuscularly). The animal's head was then secured in a head holder. Strychnine was applied to the cortex by placing thereon pieces of filter paper, 1 to 4 sq. mm., moistened with 3 per cent solution of strychnine colored with toluidine blue. All records of electrical activity were made by means of six channel Grass amplifiers and ink writers. Thirty-six electrodes—each a silver wire ending in a small silver ball—were placed on the cortex. These were arranged in groups of 6 to facilitate switching and the activity of all 36 loci was recorded before and several times after each local strychninization.

The identification of the strychninized area was made by pricking or slitting it with a sharp knife at the time the strychnine paper was removed, fixing the brain in alcohol, sectioning, and staining with toluidine blue. For the certain cytoarchitectonic identification of areas 2s and 19s with Brodmann's 2 and 19 respectively we are indebted to Dr. Gerhard von Bonin.

RESULTS

Application of strychnine to any gray mass causes large sudden voltages

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† Deceased June 9, 1940.

‡ National Research Fellow, 1940–1941.

§ We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

to appear in all places where the axones, or collaterals, of cells in the region strychninized terminate. These sudden voltages—called strychnine spikes—serve, therefore, to reveal the directed functional relation of various cortical areas.⁹

Figure 1 is a new map of the sensory cortex and adjacent regions. It is made on the basis of the previous maps of the sensory cortex and has been extended in conformity with the new findings. The numbers indicate the cytoarchitectonic areas of Brodmann¹ and the Vogts¹⁶ but are simplified in the face region where no distinction could be made on the basis of organiza-

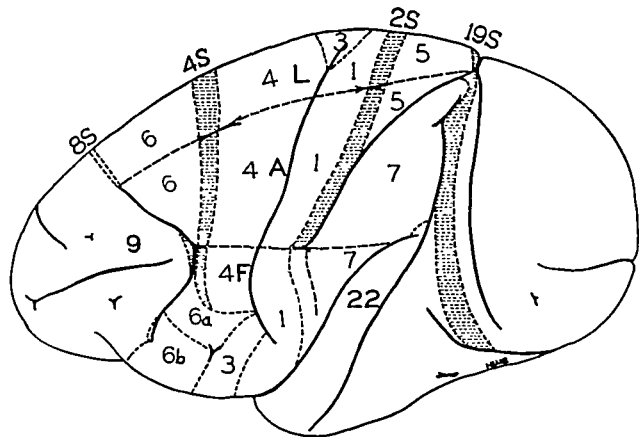


FIG. 1. *Macaca mulatta*. Diagram of sensory and adjacent cortex and convexity of hemisphere. Areas 6, 4s, 4, 3, 1, 2s, 5 and 7 constitute the sensory cortex. Letters L, A and F indicate the functional subdivisions for leg, arm and face respectively. ===== indicates areas from which suppression of electrical activity of the cortex can be obtained. Of these areas 8s and 19s are immediately adjacent to the sensory cortex but not part of it.

tion between 6b α and 6b β . The subdivisions for leg, arm and face are indicated by the appropriate letters.

Since this work was completed a new finding of interest with respect to the sensory cortex has been obtained in experiments on the occipital lobe in which connection it will be fully reported later. In area A7 immediately below the sulcus interparietalis and sometimes confined to it lies a narrow strip of cortex which is fired from the occipital lobe. To date it is the only part of the sensory cortex fired by strychninization of the cortex anywhere outside the sensory cortex.

Insofar as the results merely confirm previous findings they will not be reiterated except by schematic representation in the conclusion. All results to be considered are of one of two types, namely the occurrence of the large

sudden voltages, called strychnine spikes from their appearance in the record, or the transient diminution or disappearance of electrical activity produced by strychninization of restricted areas and called the suppression of electrical activity. (i) Local strychninization of area 9 neither produces strychnine spikes (*i.e.*, "fires") nor reduces the electrical activity of (*i.e.*, suppresses) any area of the sensory cortex. Occasionally its strychninization is followed by increase of activity of several cortical areas but this occurs so irregularly and its site is so various that it must depend upon other variables than those controlled in these experiments. Except for this, the only effect of strychninization of area 9 is the firing of area 9. (ii) Local strychninization of area 8s fires area 8s locally and suppresses the electrical activity of the entire hemi-

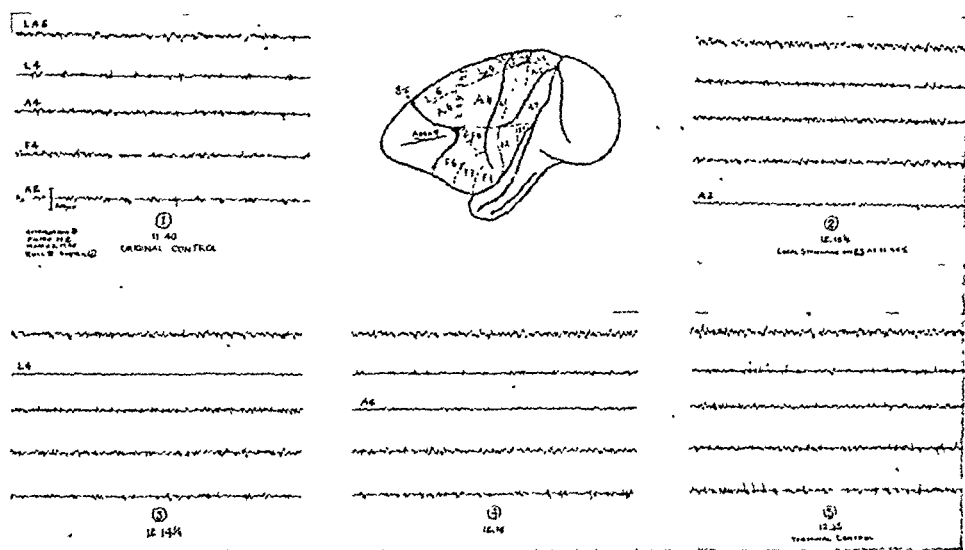


FIG. 2. March 2, 1940. *Macaca mulatta*. Dial. Strychninization of 8s showing suppression first seen in A2 later in L4 then in A4.

sphere.^{5,12} The suppression appears first in regions nearest to area 8 and sweeps across the cortex. It usually begins 7 to 12 min. after the application of strychnine, lasts several minutes and requires about 15 to 20 min. to cross the sensory cortex. Figure 2 shows the records of such a suppression. (iii) Nothing new has been discovered with respect to strychninization of area 6 except that its anterior margin has several times been found well forward in the concavity of the sulcus arcuatus. (iv) Strychninization of 4s (the "strip" of Marion Hines^{13,14}) has twice been accomplished without impinging on area 6 or area 4. (This was done only after experiments on the chimpanzee had shown the simplified picture which one might hope from a pure 4s strychninization.) It fired itself locally and no other area and it produced a suppression like that obtained from area 8. (v) Local strychninization of area 4 so far has yielded no new findings although on the basis of the finding on

the chimpanzee⁶ one would have expected them. (vi) Local strychninization of area 3 fires itself very strongly and also areas 1, 2 and 5 but weakly. It produces very questionable firing, if any, in area 4 and perhaps (on one occasion) in area 7. (vii) Local strychninization of area 1 fires itself and areas 2, 5 and 7 strongly. It also fires weakly area 4 and possibly area 4s. (viii) Local strychninization of area 2s in the leg- or arm-subdivision fires itself only locally and gives a suppression of the entire cortex like that seen after strychninization of 8s and 4s. In the face area the attempt to strychninize area 2s has usually been unsuccessful and when successful has produced at most a weak suppression. (ix) No new findings have appeared after strychninization of area 5. (x) The same may be said of strychninization of area 7. (xi) Between area 7 and the first temporal sulcus there is sometimes, but not always, demonstrable a narrow triangular area—area 22—local strychninization of which fails to fire or suppress any part of the sensory cortex. (xii) Immediately posterior to the sensory cortex medial to the sulcus interparietalis and either separated from it only by the medial prolongation of area 22 or only by the first temporal sulcus lies area 19s strychninization of which produces a suppression like that obtained from 8s, 4s and 2s.

Two observations concerning the variations of the areas with respect to anatomical landmarks deserve mention.

(i) Area 8s, identified by the elicitation of eye movements and by strychninizations which yield suppression of electrical activity of the cortex, extends from the medial aspect of the hemisphere^{12,15} laterally between areas L6 and 9 to almost the superior end of the medial limb of the sulcus arcuatus. From this point downward it usually passes into the anterior lip of the sulcus to emerge again only for a short distance as it crosses the concavity of the sulcus. However, it has appeared in one animal on the medial lip of the upper ramus, disappeared into the depths to reappear on the surface posterior and lateral to the sulcus in what is normally F6b; whereas, in another animal it crossed the concavity ending anterior to the tip of the inferior ramus.

(ii) Even greater variation occurs at the posterior margin of the sensory cortex above the sulcus interparietalis, where exploration is rendered difficult by a complex of veins in the vicinity of the sulcus parieto-occipitalis. Here precise localization can be obtained in only a small percentage of hemispheres, yet even these few disclose that area 5 sometimes extends into the sulcus parieto-occipitalis, and area 19 appears on the occipital lobe posterior thereto. In extreme cases area 5 stops short and area 19 appears in the parietal cortex and may even be separated from the sulcus parieto-occipitalis by an area a millimeter or two wide exhibiting properties of an area normally found in the occipital lobe. This latter arrangement resembles closely the relation to landmarks most frequently encountered in the chimpanzee.

DISCUSSION

Two aspects of the present findings should be mentioned. The first of these is the existence of the suppressor bands 8s and 19s which bound the

sensory cortex, except for area 22 occasionally coming to the surface in front of the first temporal sulcus. As the animals used in this experiment were frequently used also for studying cortico-striatal projections,^{5,8} it is possible to be certain that area 8s, like area 4s, projects to the nucleus caudatus. As it has already been shown that this projection is responsible for suppression from 4s, it seems only reasonable to conclude that it is likewise effective in the case of 8s, particularly in view of the finding that strychninization of the nucleus caudatus can itself produce suppression by blocking thalamo-cortical reverberations. As yet it has not been possible to demonstrate similar projections from areas 2s and 19s.

Since this statement was made Drs. Hugh Garol and Percival Bailey, working in this laboratory, have shown that strychninization of 2s does fire far posteriorly into the horizontal portion of the nucleus caudatus. This finding makes it advisable to expect connections of 19s to still more caudal portions of the nucleus caudatus.

There are, therefore, on the convexity of the hemisphere of the monkey four suppressor bands, two within and two just without the sensory cortex and all four are alike in their failure to fire other portions of the sensory cortex. Thus it resembles the chimpanzee's functional organization.

The second point requiring comment concerns the clarification of the findings in strychninization of 4s and 2s. In the case of 4s, the pure picture differs from the previous mixed (which we had mistaken for pure) merely by the omission of firing elsewhere. In the case of 2s (which we, lacking histological control, had tentatively called 1) the change is more complicated. Reference to the previous diagram of functional organization⁷ shows that areas 2 and 5 had no difference as to other regions fired from them, a finding out of line with those for all other areas strychninized. The difference which now appears is that the area we had considered to be 5 failed to fire other parts of 5, whereas 2 did so. It is now clear that both were really area 5, and that within 5 the firing is more restricted in the most posterior portion. In the paper mentioned above all mention of leg or arm 3 was omitted, for it is difficult to strychninize it without touching area 1 and the difference in firing of other areas from 3 and 1 is not clear enough to be absolutely certain of the results. The same may be said of pickup electrodes intended for area 3, *i.e.*, disturbances from 1 may be included. It seems therefore wisest to omit area 3 from the schematic representation of the findings except for the face region. When these modifications are made the new scheme and the new map bear a still more striking resemblance to that of the chimpanzee, at least so far as the leg and arm regions are concerned.

As already stated, the work on the chimpanzee gave clues as to what might be expected in the monkey, for, being larger, the bands of the chimpanzee's cortex were more easily strychninized separately. Lest the work on the chimpanzee be thought to have been unduly influential in producing the similarities noted above it should be emphasized that, in the monkey, repeated careful attempts to distinguish two bands in area 4 and any subdivision into bands of area 5 or 7 have failed completely, except for the more

restricted firing in the posterior part of area 5. Whether the failure to find these differentiations is due to their non-existence or to the small size of the area to be subdivided cannot be determined by the procedures here employed. On the other hand, if one considers the threshold to electrical stimu-

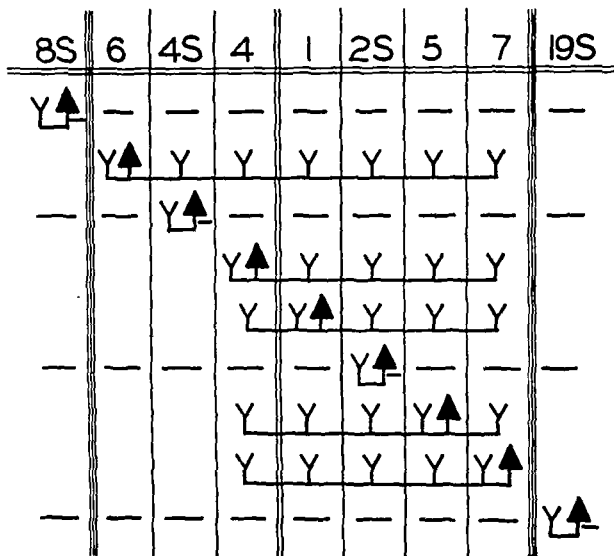


FIG. 3. Functional organization of sensory and adjacent cortex of leg- and arm-subdivisions. Solid triangle indicates location of strychnine; Y, the appearance of strychnine spikes; —, the suppression of electrical activity. Diagram indicates the maximum results obtained by strychninizations confined to the areas indicated. Double lines indicate sulcus centralis; triple lines, the confines of the sensory cortex.

lation, area 4 of the monkey is divisible into two bands, and, as excitation by strychninization remains generally more nearly confined to its site than does that by electrical stimulation, one would expect that a difference of functional organization of these bands in 4 would certainly be discoverable if it exists. It was hoped that the somewhat larger brain of a large mangabey might have sufficiently larger areas to show separate bands in areas 5 and 7 but it failed so to do. In this manner and in these respects the monkey's brain seems to lack differentiations found in the chimpanzee and here the maps remain significantly dissimilar.

SUMMARY

The accompanying schematization (Fig. 3) indicates only those findings

which are well established in the monkey and summarizes the results of the present and previous investigations of the functional organization of the sensory and adjacent cortex.

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ERNST THEODOR VON BRÜCKE

1880-1941

THE EDITORS of the *Journal* deeply regret to announce the sudden death of Ernst Theodor von Brücke on the night of June 11-12th. Dr. von Brücke had been a member of the Advisory Board of the *Journal*, representing neurophysiology in Austria, since the *Journal's* inception in January 1938.

Ernst T. von Brücke, born October 8, 1880 in Vienna, was for many years Professor of Physiology at Innsbruck, where he enjoyed the affectionate regard and high esteem of pupils and colleagues alike. As a teacher his eminence was attested by the crowded attendance at his lectures even when attendance was optional. In research he was a scholarly and impartial judge of evidence, a skilled technician and an eager and enthusiastic worker. Many important and fundamental papers on the nervous system came from his laboratory.

The honesty and sincerity of his quest for truth was shown by his readiness and even eagerness to accept criticism, to concede errors in interpretation and to revise his views as soon as new evidence indicated the need of revision. He wanted the truth regardless of whether or not it was at variance with his previous opinions.

A true scientist, he loved his work and his laboratory, and when on the Nazi conquest of Austria in 1938 he was dismissed from his professorship and ordered not to re-enter his laboratory, his depression was profound and pathetic. Enforced idleness and separation from the work he loved were almost more than he could bear. Even Nazi students, meeting him in the street, told him of their regret that they could no longer enjoy his teaching.

An appointment as Research Associate in Physiology in the Harvard Medical School was arranged for him, and he came to America in August 1939. Entering enthusiastically on a new program of research, he at once began to achieve noteworthy results in the study of the refractory phase of nerve and the effect thereon of prolonged stimulation—a problem which his earlier experiments with Field had rendered controversial and in need of clarifying. These researches have already resulted in the publication of one paper, while a second is in press in this *Journal*.

In recent weeks he had begun a new series of experiments on reflex inhibition, requiring a rare combination of skilled techniques. Significant results had already begun to appear. It was my privilege on June 11 to confer with him on these observations and to watch him performing one of the most brilliant experiments I have ever seen. He went home, apparently in the best of health and spirits, and happy in the progress of his new research. Early the next morning he died peacefully in his sleep.

High as was his standing as a scholar and investigator, he will be remembered chiefly by those who knew him for his extraordinarily kind and sympathetic nature and his nobility of spirit.

ALEXANDER FORBES

Harvard Medical School
Boston, Mass.
June 16, 1941

CEREBRAL BLOOD FLOW AND pH IN EXCESSIVE CORTICAL DISCHARGE INDUCED BY METRAZOL AND ELECTRICAL STIMULATION

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INTRODUCTION

THE FUNDAMENTAL MECHANISM involved in the initiation of paroxysmal excessive discharges of the cerebral cortex has been investigated recently from several avenues of approach. Epileptiform discharges of the cerebral cortex, either experimentally induced by electrical stimulation or by convulsant drugs in animals, or as associated with epileptic seizures in man are accompanied by an increase in circulation through the discharging area, as shown by Gibbs (1933), Gibbs, Lennox, and Gibbs (1934), and Penfield, von Sántha and Cipriani (1939). The change in circulation appears, however, to be secondary to the increase in neuronal discharge. Since a change in cerebral circulation has never been observed to precede the onset of excessive neuronal discharge, but always to follow evidence of a convulsion, Penfield, von Sántha and Cipriani conclude that circulatory changes probably do not play a part in the immediate initiation of epileptiform activity. No direct evidence has been given pertaining to the mechanism of this increase in blood flow but the release of CO₂ or acid metabolites of activity has been suggested as well as a possible local accumulation of acetylcholine. Simultaneous records of local blood flow and the electrical activity of the cortex have not been reported.

Simultaneous measurements of electrical activity, pH and DC voltage changes of the cortex have led Dusser de Barenne and colleagues (1937, 1938 and 1939) to conclude that increased pH as well as a change toward negativity of cortical DC level are associated with facilitation of cortical response. Facilitation was manifest by increase excitability to electrical stimulation with increased after-discharge as well as increased spontaneous electrical activity.

Bonnet and Bremer (1937), Moruzzi and Bremer, (1938), Moruzzi (1938, 1939) and Miller, Stavraky and Woonton (1940), point out that acetylcholine may reproduce a condition of facilitation of the cortex similar to that caused by intense electrical stimulation. They suggest the possibility of acetylcholine playing a part in the normal facilitation process, or in the words of Bremer, (1938) to raise the "tonus corticale." Alterations in cerebral circulation have not been considered with these other factors for facilitation. The present study is concerned with the relationship between focal alterations in blood flow and pH with excessive cortical discharge in response to electrical stimulation and convulsant drugs.

METHOD

Experiments were carried out on 23 cats under light ether anesthesia with curare and artificial respiration or, in some experiments, with dial anesthesia. A bilateral craniotomy was performed exposing the right and left sigmoid gyri. Most records were taken from the posterior sigmoid gyrus of one side with electrical stimulation of the homologous area of the other hemisphere. Recording leads were applied immediately following the opening of the dura and special care was taken to maintain optimal conditions of temperature and humidity throughout the experiment.

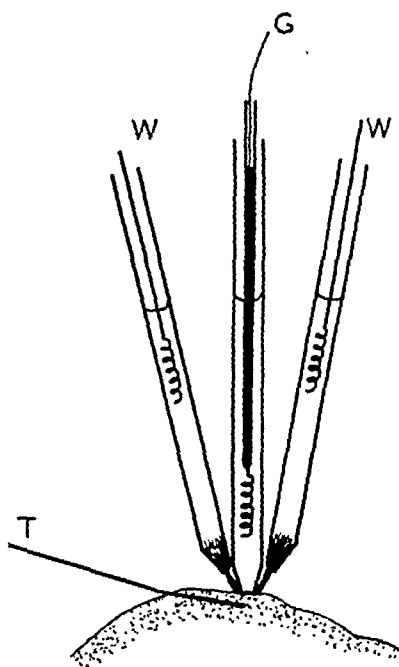


FIG. 1. Schematic diagram of arrangement of electrodes on the cortex. The silver silver-chloride wick electrodes (W) are placed on either side of the glass electrode (G) with the thermocouple (T) placed so that its tip lies just beneath the glass electrode. The wick and glass electrodes are supported in a spring holder to minimize changes in pressure from movements of the brain.

The electrical technique for the simultaneous recording of focal cerebral blood flow, pH and electrograms together with femoral blood pressure has been described in a previous report (Jasper and Cipriani, 1940).^{*} In addition to these measures a similar technique was employed in the present study for the recording of DC voltage changes at the site of the pH glass electrode. The disposition of the various recording devices on the cortex is shown in Fig. 1. Silver silver-chloride wick electrodes were used as non-polarizable leads for DC voltage measurements, one of the wick electrodes being used in common with the reference lead for pH measurement. This made it possible to record the DC voltage across an area of the cortex as nearly as possible the same as that used for the pH measurement. The wick electrodes were in contact with the glass electrode on either side, as shown in Fig. 1.

In preparing the cortex the dura was removed and the pia perforated or partially removed just beneath the point in the post sigmoid gyrus where the glass electrode was to be applied. This was found advisable for the pia caused some delay in the recording of cortical pH changes as determined by records taken before and after its incision. The thermocouple for blood flow was placed about 1 mm. beneath the surface of the pia with the point exactly beneath the glass electrode.

In order to estimate the amount of temperature correction for the pH measurement the thermocouple was used to measure cortical temperature in some of the experiments. Since, however, adequate temperature readings of the cortex immediately at the point of application of the pH electrode were not taken throughout all of the experiments, the absolute values of our pH measurements are subject to an experimental error estimated as of the order of ± 0.03 units. The temperature coefficient of our glass electrodes was 0.007 pH per degree C. and showed no significant variation from one electrode to another.

Control studies turning on and off the heater element of the thermocouple have shown that the heat of the thermocouple itself does not affect appreciably the pH measurements. This would also indicate that temperature changes beneath the pH electrode due to alterations in blood flow are probably insignificant in affecting pH measurements.

The latency of the glass electrode was determined by placing it in a phosphate buffer of pH 8.0 and injecting a drop of the same type of buffer pH 7.5 just beneath the glass electrode with a syringe. A second record was taken with an additional electrode in the solution to provide a signal for the instant at which the drops came in contact with the

^{*} We are greatly indebted to Dr. Leslie Nims for teaching us the glass electrode technique as applied to the exposed cortex.

surface of the liquid. With injections immediately beneath the glass electrode, the latency of the recorded change in pH was about 0.08 seconds. Latencies as long as 0.2 and 0.3 sec. were obtained when a drop of the buffer solution was placed beside the glass electrode in the solution. The diffusion time to the glass electrode may add to this delay in actual experiments as demonstrated by these calibrations. No satisfactory measure of this factor was made. Its importance is hard to estimate. Electrograms were taken from as nearly as possible the same cortical area as for the pH measurements. Electrical activity may be recorded from deeper layers, however, causing a delay in pH of that area as recorded from the surface. The latency of the thermocouple for changes in temperature was determined by exposing a light to the tip of the thermocouple beside which was placed a photoelectric cell to signal onset of light. The latency to initial perceptible change was 0.17 sec. The amplifiers used for recording possessed sufficient in-phase degenerative feed-back so that completely independent records were obtained from each amplifier, as proven by repeated control tests.

Electrical stimulation was carried out by means of a thyratron stimulator at a frequency of 60 cycles per sec. through bipolar silver electrodes with a separation of 3 to 5 mm. Stimulation was always carried out on the contralateral cortex to avoid the effect of passing current through the recording electrodes. Both bipolar and monopolar leads were used for the electrograms either with separate silver electrodes or by using the same wick electrodes as for the DC measurements. The essential feature of the technique employed for all these experiments is that simultaneous records of focal changes in pH, blood flow, DC voltage and electrical activity were recorded in ink on a single strip of paper. Direct measurements of the temporal sequence of events was thereby made possible. In the experiment where DC voltage was substituted for blood pressure, blood pressure was read from the mercury manometer and inserted on the record by hand.

EXPERIMENTAL RESULTS

Adrenalin

Effect on blood flow. Injection of adrenalin intravenously (0.2 to 0.4 cc., 1/10,000 solution) was used to test the reliability of the thermocouple for changes in blood flow. The invariable rise of blood pressure so produced was accompanied by a constant increase of blood flow through the cortex, thereby furnishing a good test of the sensitivity of the thermocouple as well as the direction of change.

Effect on electrogram. Under the conditions of our experiment there was either no perceptible change or a slight increase in the electrical activity of the cortex following the administration of adrenalin, if the animal had not previously received an injection of metrazol, and if the pH level of the cortex was relatively normal at the time of the injection (Fig. 2). Following an injection of metrazol, however, after the metrazol discharge had ceased, an injection of adrenalin would cause its reappearance. Also, if a dose of metrazol had been too small to produce large discharges of the cortex, the subsequent injection of adrenalin would precipitate the typical metrazol activity. This potentiation of metrazol discharge with adrenalin has been observed by previous investigators (Hall *et al.*, 1938).*

* Previous injection of adrenalin did not affect the convulsive threshold to metrazol. For this reason as well as because of the fact that adrenalin itself produces no such change in brain waves, it may be assumed that adrenalin mobilizes metrazol from some other organ which has taken it up. It has been shown that the liver is capable of retaining for some time large amounts of metrazol (Esser and Kühn, 1933). It is quite probable that metrazol taken up by the liver is mobilized into the blood stream by adrenalin analogous to the mobilization of glucose from glycogen stores.

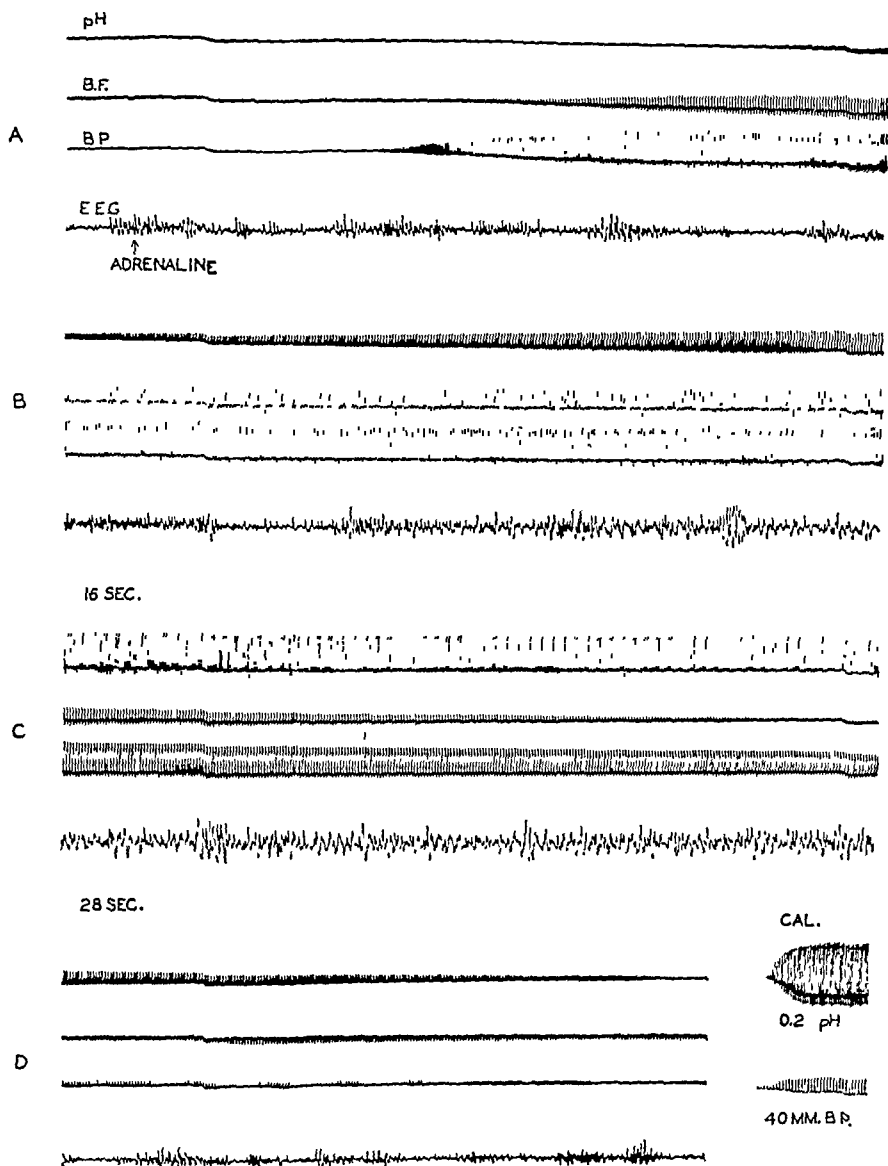


FIG. 2. The effect of adrenalin on focal cerebral pH, blood flow (B.F.), systemic blood pressure (B.P.), and cortical electrogram (E.E.G.). Initial pH balanced at about 7.2. Adrenalin injected into saphenous vein was followed in 9 sec. by a rise in B.P. and in 14 sec. by a rise in local cerebral blood flow (A). The pH did not begin to rise until about 25 sec. following the injection (B). Maximum pH rise was about 1.4 which was maintained after B.F. and B.P. had returned to normal (C and D). All records returned to preinjection level about 150 sec. following the injection (end of D). There was a moderate increase in the electrical activity of the cortex during the rise in pH. Each full line of record equals 30 sec.

Effect on pH of cortex. Under the conditions of our experiment the most common effect of adrenalin was to increase the pH of the cortex. This increase in pH usually followed the increase in blood flow, and often persisted longer than the increase in blood flow (see Fig. 2). The change in pH would seem to be a direct result of the removal of acid metabolites by the improved circulation.

Dusser de Barenne, Marshall and Nims (1938) demonstrated that the pH of the interstitial fluids of the cortex does not always follow exactly the pH of arterial blood. No records were taken of the pH of arterial blood but it seems logical to assume that, under most conditions, the cortical pH was somewhat below that of the blood so that an improvement in circulation tended to its increase. There were a few exceptions to this which probably signify that under certain conditions the balance between the pH of cortex and blood is so close that an increase in circulation causes little change in cortical pH, but the usual effect was an increase.

Metrazol

Intravenous injection of metrazol (pentamethylenetetrazol) was used as a convenient and dependable method of inducing abnormal and excessive discharge of the cortex. Even though the type of large amplitude electrical discharges obtained from the cortex, and the type of convulsions produced simulate those observed in certain forms of epilepsy, it cannot be assumed with certainty that the mechanism of the metrazol seizure is the same as that of epileptiform discharges produced by other methods.

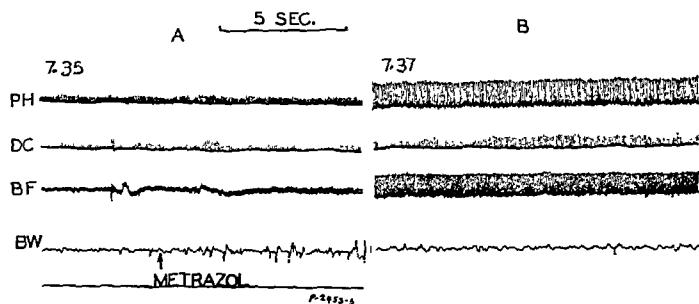


FIG. 3. Metrazol subliminal for cortical discharge but causing increase in blood flow and pH. In A, pH is shown (upper line) approximately balanced at 7.35 when metrazol (0.5 cc. of 10 per cent sol) was injected. DC voltage between the two wick electrodes was approximately balanced as shown in second line and blood flow (B.F.) on the third line. Electrical activity between the two wick electrodes (A.C. recording without "chopper") is shown in fourth line. Five minutes later (B) the pH increased to 7.37 with a small rise in blood flow (third line) and little change in D.C. (second line) or in electrical activity (lower line).

Metrazol was given by rapid intravenous injections in doses of 0.020 to 0.050 g. per kg. body weight.

Subliminal doses. Doses too small to affect cortical activity produced marked changes in blood pressure, blood flow, and pH. The increase in blood pressure was followed by an increase in blood flow and this, by an increase in pH (see Fig. 3). Further demonstration is here provided of the effect of increased blood flow on cortical pH, which must be kept in mind in evaluating changes due to supraliminal metrazol injections.

Supraliminal doses. With supraliminal injections of metrazol a sharp and large increase in electrical activity was in every instance the first ob-

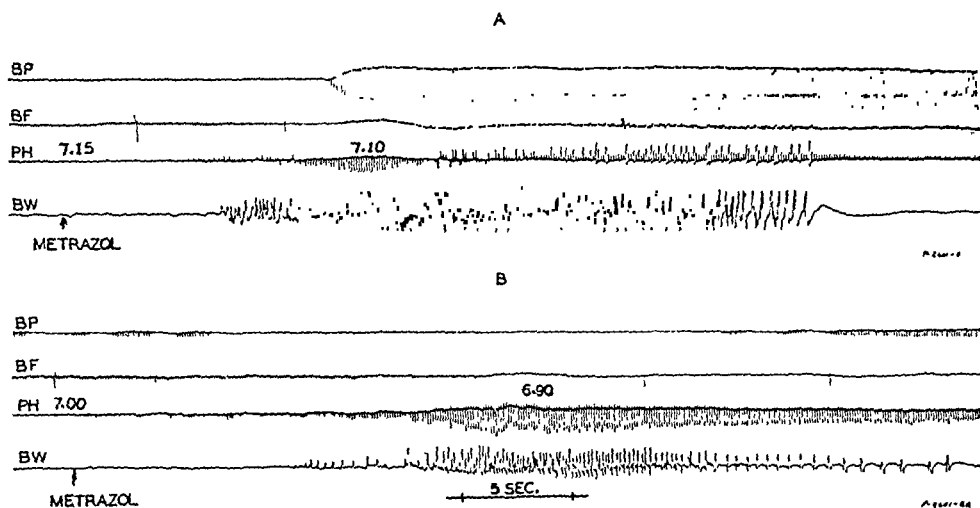


FIG. 4. Sequence of changes in blood pressure (BP), blood flow (BF), pH, and cortical electrogram (BW) following the injection of metrazol (0.6 cc. 10 per cent sol.). A. First injection, showing initial pH decrease (7.15 to 7.10) and compensation with pH increase as blood flow rises (blood pressure reaction not typical). B. Third injection about one hour later, showing only pH decrease (7.0 to 6.90) with no blood pressure or blood flow change.

servable change to occur. Following closely upon this initial burst of large amplitude waves there were changes in pH, blood flow and blood pressure. Changes in pH usually preceded slightly the change in blood flow in the majority of the most reliable experiments, but a simultaneous change in pH and blood flow as well as a slight precedence of blood flow change was observed in some instances. In a typical experiment the metrazol discharge appears in the electrogram 7-10 sec. following injection in the saphenous vein. About 2-3 sec. later the pH is decreased and blood flow begins to increase. Then the pH may return to normal or go relatively alkaline if there has been a sharp rise in blood flow.

The first pH change following the metrazol discharge was usually in the acid direction. If there was a prompt increase in blood flow the pH would revert to the alkaline side and then either return to the preinjection level, or go again "acid," depending upon the amount of compensatory blood flow

increase. With no marked change in blood flow, only an acid change in pH would be observed for the duration of the excessive neuronal activity. These changes are illustrated in Fig. 4A and B.

The initiation of the metrazol discharge appeared therefore to be due to an exciting or facilitating action of metrazol upon nerve cells apparently not related to initial changes in pH or blood flow. The latter changes appear as a result of excessive activity induced by metrazol. This is assuming a negligible diffusion lag in the pH system. The fact that the initial pH change is usually in an acid direction is a strong argument for its being due to the initial increase in neuronal activity. Some of the initial changes in blood flow may of course be due to direct effect of metrazol on the cardiovascular system. This would complicate the observed relation between pH and blood flow as related to metabolites from excessive neuronal activity.

There was an *increase* in cortical blood flow parallel to an increase in blood pressure following metrazol injection in the majority of experiments. Little change in blood flow or a decrease was observed only when the animal was in poor condition as when repeated doses of metrazol had been given, or the initial pH was low (7.0 or less) a condition often associated with low blood pressure. This has been confirmed by direct observations of cerebral vessels in experiments of Erickson and Dancey (unpublished).

There seemed to be a delicate interaction between blood flow and pH, apparently for the maintenance of constant pH of interstitial fluid in the presence of abnormal neuronal discharge. With prolonged and excessive cortical discharge this homeostatic mechanism would often fail to keep the pH at a normal level, so that a continued increase in acidity would result. After several metrazol injections the pH was not uncommonly found to go below 7.0. Values above 7.1 were maintained when the animal was in good condition.*

Spontaneous cyclic discharge. In many experiments, following the initial metrazol discharge lasting 10 to 20 sec.; cortical activity would suddenly disappear as though turned off with a switch. Then after a quiet period of 15-25 sec. it would suddenly reappear spontaneously, in all its former vigor. The duration of the active and quiet periods varied considerably but this periodic alternation in violent activity and quiescence might continue for over an hour without additional metrazol if the cortex was in good condition. This phenomenon afforded a good opportunity to study the relation between cortical pH and cortical activation.

Blood flow showed no periodic change associated with the cycles of electrical activity. There was usually a steady *increase* in blood flow at first until a maximum was reached.

* The pH of the cortex was found to be a reliable index of the general condition of the animals. In animals which had suffered loss of blood or operative shock a low initial pH was always obtained. The death of the animals was often preceded by a continued decrease of pH well below 7.0 even though the electrical activity of the cortex was still present, though depressed.

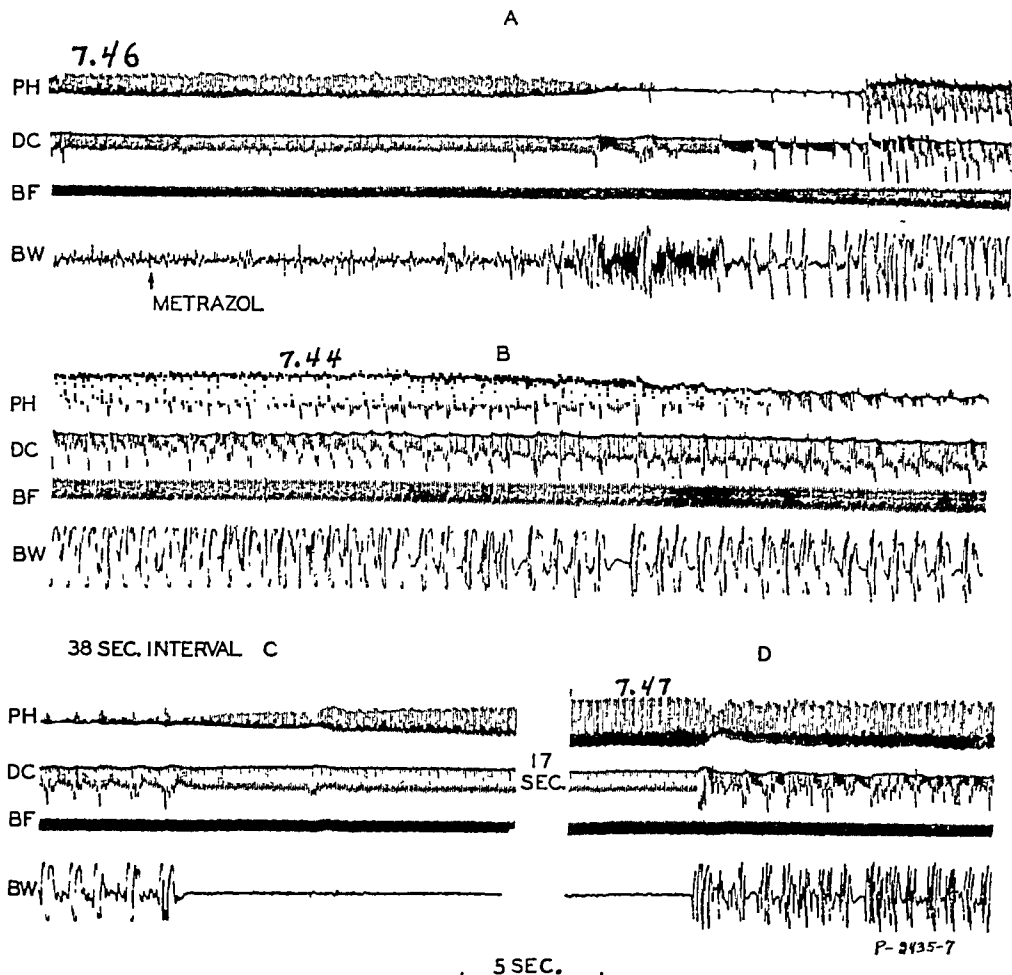


FIG. 5. Cyclic metrazol discharge (BW) with associated changes in pH, DC voltage, and local blood flow (B.F.). ("Chopper" records were not balanced completely at beginning.) Initial pH calculated to be about 7.46 before metrazol. Concurrent with the onset of metrazol discharge in lower line of A the pH dropped to reach 7.44 in B. With the increase in blood flow shown in third line (B) the "acid" drift of pH was arrested and partially compensated during continued metrazol discharge (end of B and for 38 sec. following). Then the metrazol discharge suddenly stopped spontaneously (C) after which the pH slowly rose to 7.47 when another prolonged metrazol discharge began to repeat the cycle. These pH changes seemed independent of DC voltage changes.

There was an acid drift in pH during the active phase of the cycle and an alkaline drift during the quiet period. If the blood flow was increased sufficiently during the active phase the pH would show an alkaline drift following the acid drift *during the cortical discharge*. The cortical discharge would suddenly stop, in this case, when the cortical pH was more alkaline than during the height of activity (see Fig. 5). Relative acidity could not be called upon to explain this sudden cessation of cortical activity. Change in pH seemed to

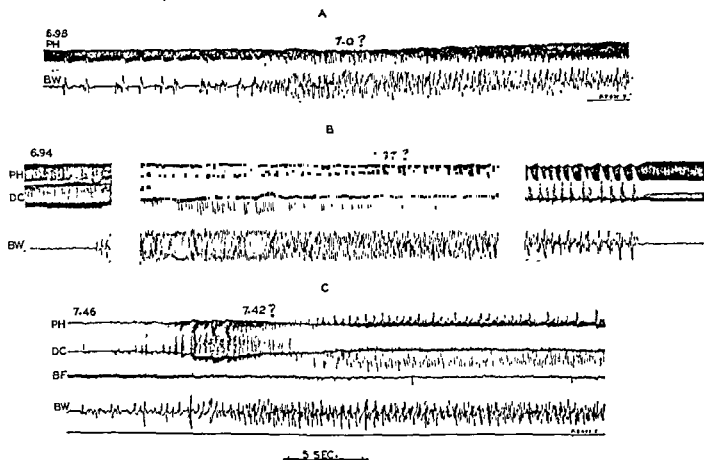


FIG. 6. Apparent "alkaline wave" due to artifact of DC component in cortical electrogram. A—Slight interrupted "alkalinity" with most rapid phase of electrogram. B—Change in DC simultaneous with apparent alkalinity of pH during rapid phase of electrogram and slow waves of "pH" at end due to slow DC changes in electrogram. C—Parallelism of DC and "pH" records during metrazol discharge. In this case the initial apparent "pH" change was "acid."

follow changes in blood flow but did not show any clear effect on the metrazol discharge. The cortical pH at any given moment was a function of the amount of neuronal activity which caused a decrease and the compensatory increase in blood flow which tended to counteract the developing "acidity."

After the cortical blood flow had reached its maximum the pH would then begin to show a steady acid drift throughout the interrupted trains of metrazol waves. If the cortical activity was continued long enough the pH might reach values below 7.0 before an appreciable diminution in the violence or duration of periodic discharges was detected.

Initial "alkaline waves." An apparent initial "alkaline wave" with the onset of metrazol discharge was observed in a few experiments, confirming the observation of Dusser de Barenne and McCulloch (1939). This initial "alkaline wave," when it did occur, seemed to be related to DC voltage components of the large cortical discharge. In some instances it may have been associated with DC polarization changes. An example of such an alkaline wave is shown in Fig. 6A and B. It will be noted that the alkalinity is associated with the most rapid phase of cortical discharge.* With the more rapid

* Dusser de Barenne and McCulloch (1939) also associate the "alkaline wave" with the rapid phase in electrocorticograms but interpret this as meaning greater facilitation. It appears to us that this might also produce a greater voltage artifact.

recording system used, the pH record shows an irregular interrupted appearance. With a slow moving galvanometer recording the pH changes, this would appear as a smooth alkaline wave. It seems, therefore, to be an artifact due to electrical disturbances of cortical origin.

In order to control this factor, simultaneous records were taken of the DC voltage changes from two wick electrodes placed on either side of the glass pH electrode, using one wick for the common reference electrode of the pH system as well. As shown in record B of the above figure, there was occasionally a definite relationship between DC voltage and changes in pH. This was not always in an alkaline direction however, as illustrated in Fig. 6C. It depended upon whether the DC voltage change occurred nearer the reference electrode or nearer the glass electrode, there being an equipotential point between them.

Further control on the DC voltage artifact was made by disconnecting all the leads except those measuring pH to rule out the possibility of interference between the recording systems. The voltage artifacts appeared even though only pH records were being taken, which showed that they originated at the cortex and were not in the recording apparatus. Also, when a 1-3 per sec. alternating current signal was placed on the two wick electrodes instead of the brain potentials, variations in the pH record could be induced artificially by voltage changes comparable in amplitude to those produced by the brain itself during a metrazol discharge. The DC artifacts were of the order of 1 to 2 mV. in magnitude or an equivalent pH of 0.02 to 0.04 units. We believe that these few observations of a small initial alkalinity with the onset of metrazol discharge can be explained on the basis of DC artifacts.*

The late alkaline waves, mentioned above, following an increase in blood flow are of larger amplitude (up to 0.36 pH units with large increase in blood flow) than could be produced by DC artifacts and are apparently related to the increased circulation. Changes of this order of magnitude were observed by the action of adrenalin alone.

Electrical stimulation

It was not possible to determine with accuracy the sequence of pH and blood flow changes following electrical stimulation, due to the large artifacts introduced into the records by the stimulating current. Records of blood pressure and blood flow were devoid of this artifact, but the pH and brain potential records were affected because of their high input resistance. Our observations will be confined mostly to changes associated with after-discharge phenomena following intense stimulation.

We were able to confirm the evidence of von Sántha and Cipriani (1937)

* It must be kept in mind that the alkaline waves observed by the Yale investigators were in response to monobromide of camphor rather than metrazol, and were present in animals under dial anesthesia. It appears clear however from our experiments that initial alkalinity played no part in the onset of the metrazol discharge. Also the occasional "alkaline waves" observed by us were small when not associated with increased blood flow, a factor not controlled in the Yale experiments.

that there is a focal increase in cerebral blood flow following local activation of the cortex from nerve impulses conducted over neuronal pathways from a distant area subject to electrical stimulation. In some instances this focal change in blood flow due to indirect stimulation was followed by a change in blood pressure (see Fig. 7). When there was marked increase of blood flow the pH either showed very little change or a slight alkaline drift. Following several intense stimulations, either with or without prolonged electrical after-discharge, the change in blood flow would be slight and the pH would show an acid drift increasing with successive stimuli. The acid drift was more marked when a long after-discharge was present.

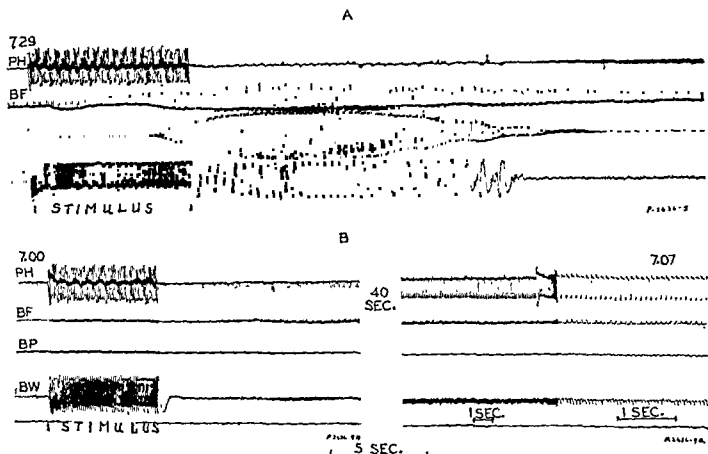


FIG. 7. Electrical stimulation of left post sigmoid gyrus with records of pH, blood flow, and cortical electrogram from the right post sigmoid gyrus. Blood pressure (BP) is from the femoral artery. Stimulus artifact appears in pH and cortical electrograms only. A—Stimulus at normal pH with rise in blood flow and blood pressure followed by after-discharge. The increased blood flow is adequate to maintain a constant pH in this example. B—Stimulus at low pH causing low voltage high frequency after-discharge, increase in blood flow and pH.

A consistent alkaline drift in pH was noted in a few experiments in which the after-discharge was of an unusual form, namely low amplitude, decelerating, rapid waves, as shown in Fig. 7B. In this example the initial pH of the cortex was low so that even a slight increase in blood flow was apparently sufficient to produce an alkaline drift of the pH. This low amplitude discharge may be associated with the production of less acid metabolites than the more violent discharge. Here again pH and blood flow changes appear to be results of local activation through nerve impulses from the

contralateral hemisphere. Changes in pH and blood flow do not seem to play an essential role in the *initiation* of abnormal neuronal discharge.

Local application of strychnine and metrazol

Local application of 3 per cent strychnine solution warmed to approximately the temperature of the cortex produced the usual strychnine spikes from the area to which it was applied. These spikes first appear as isolated random discharges separated by an interval of several seconds. During these isolated random spikes no significant change in either blood flow or pH was observed. Similar results were obtained in one experiment with the local application of metrazol solution at the same concentration as used for intravenous injection.

Records of blood flow were not reliable in these experiments due to the possible change in temperature following local application. It is of interest however, that strychnine spikes can be obtained with no significant change in pH, suggesting that here also the *excitatory effect of strychnine is independent of pH changes*. This is in confirmation of the work of Dusser de Barenne and associates.

Induced changes in pH

The pH of the cortex was altered by hyperventilation, apnea, intravenous injection of 5 per cent solution of sodium bicarbonate in normal saline, intravenous injection of 1/10 normal HCl, and by inhalation of a 15 per cent CO₂ in oxygen mixture.

The extreme changes in pH were found to alter the spontaneous activity of the cortex in the direction indicated by Dusser de Barenne and his colleagues (1939).

Large amplitude brain waves have been observed to follow the intravenous injection of sodium bicarbonate associated with a rise in the pH to slightly over 7.5.

Low amplitude brain waves were regularly observed without metrazol when the pH dropped to the vicinity of 7.1 or below. There were only small changes in spontaneous electrical activity of the cortex with variations of pH between 7.1 and 7.5. Large amplitude metrazol activity was observed with variations in pH between below 7.0 to slightly above 7.5. Injections of HCl in the midst of metrazol discharge, sufficient to alter the cortical pH as much as 0.2 to 0.3 units did not alter significantly the continued discharge, if the pH did not fall much below 7.0. With pH values below 7.0, however, there was a tendency in some of the experiments for the metrazol activity to be of lower amplitude and of short duration. Since other factors varied with this low pH, such as poor cerebral circulation, a depression of the metrazol discharge with pH below 7.0 cannot be certainly attributed to a change in pH alone.

In marked contrast to the lack of effect of pH on the metrazol discharges, apnea was followed within 10-30 sec. by a complete arrest of electrical activity of the cortex even though induced in the midst of violent metrazol

discharge. Apnea was always followed by an acid drift of the cortex, but the abolition of neuronal discharge occurred 20–40 sec. before the change in pH. This delay in pH change does not seem entirely due to diffusion lag for it is longer than that following the metrazol discharge (2–3 sec.) as well as longer than the pH change with increased blood flow (2–5 sec.). The suppression of metrazol discharge seems, therefore, to be due to anoxia and not to an excess of carbon dioxide or a change in cortical pH.

Summary.—It appears that pH is not a significant factor in the control of the metrazol discharge but that an adequate oxygen supply is essential for its maintenance.*

DISCUSSION

The relative independence of the metrazol discharge from pH changes between 7.1 and 7.5 under the conditions of our experiments does not necessarily imply that cortical facilitation is not affected by pH when tested by electrical stimulation or by the amplitude of spontaneous activity as in the experiments of Dusser de Barenne *et al.* Metrazol may be such a powerful facilitating agent in itself that changes which might appear with pH are "swamped." It seems clear, however, that the facilitating action of metrazol is not mediated by a change in pH nor is it greatly affected by induced changes in alkalinity of fluids bathing the cortical tissue affected. This is at least one type of facilitation mechanism which operates without parallel changes in pH.

The observation that all of the relatively large changes in pH (over 0.1)

* An experiment on one husky tom cat with "epileptic" tendencies was particularly interesting. A single injection of 0.6 cc. metrazol caused a cyclic violent discharge in the electrogram for over one hour. Curarization was sufficient to eliminate any convulsive movements. pH at the onset was 7.30.

After 18 min. 10 cc. of N/10 HCl was injected i.v. The cortical pH was reduced to 7.20 with no effect on metrazol discharge.

At 24 min. complete cessation of respiration caused a disappearance of cortical activity in 60 sec. even though the pH dropped to only 7.16. After 90 sec. apnea normal respiration was reinstated. Metrazol discharges then reappeared in short bursts after 120 sec. when the pH was still reduced to 7.10.

Hyperventilation was then administered for 4 min. causing a rise in pH to 7.38 at which time the metrazol discharge continued unchanged. Twenty cc. HCl was then injected in the midst of violent cortical activity. This reduced the pH to 7.23 but did not stop the metrazol discharge. It was promptly stopped again after 15 sec. apnea (pH 7.15). After a second period of apnea lasting 2 min. normal respiration was reinstated. Large amplitude slow waves at about 2 per sec. appeared in the electrogram after 3 min. normal breathing, pH 7.07. Hyperventilation for 9 min. then caused a reappearance of the metrazol activity and a rise in pH to 7.48. A continuous volley of rapid metrazol discharge then appeared lasting 9 min.

A third injection of HCl (20 cc., making total of 50 cc.) then caused a rapid drop in pH to 6.99 without stopping the metrazol discharge although it became less continuous.

Hyperventilation then caused the pH to return to 7.29 at which time the metrazol discharge stopped 80 min. following the single injection of metrazol. A second metrazol injection of 0.6 cc. caused a prompt return of violent cortical discharge. The cat was then given 15 percent CO₂ in oxygen instead of air which caused a rapid fall in pH to below 6.5 again without stopping the metrazol discharge. Metrazol discharge was finally stopped 30 sec. following the beginning of terminal bleeding from the femoral artery.

in an alkaline direction were associated in these experiments with an increase in blood flow and the changes in an acid direction were associated with excessive cortical discharge, inadequately compensated for by increased blood flow, indicates that focal cerebral circulation must be taken into account in all attempts to correlate pH and cortical activity. Since increased cortical discharge of any kind tends to increase the local blood flow, thereby tending to increase the pH of this area, the observed relative alkalinity with facilitation of cortical discharge may be a result, rather than a cause of facilitation. It is highly probable, however, that alkalinity as such may lower the threshold of discharge for cortical neurones as has been shown for peripheral nerve, but that pH is an important mechanism in the physiological control of cortical excitability has yet to be demonstrated.

CONCLUSIONS

1. An increase in local cerebral blood flow brought about through the action of adrenalin, metrazol, or by metabolites of increased neuronal activity tends to increase the local pH of the cortex.

2. The vascular dilatation resulting from increased local acidity due to neuronal discharge tends to maintain a constant pH of the cortex under physiological conditions; a delicate homeostatic adjustment mechanism for normal brain function similar to that found in other body tissues.

3. Excessive neuronal activity may exhaust the circulatory adjustment to such an extent as to cause a marked decrease in cortical pH.

4. The onset of a metrazol discharge is characterized first by excessive neuronal activity, then relative acidity followed by relative alkalinity if blood flow is increased. Only acidity results if blood flow is not increased. Increased blood flow with metrazol discharge is regularly observed when the animal is in good condition; failure of increase or a decrease being a sign of deficient reactivity of the cardiovascular system.

5. Apparent small initial "alkaline waves" concomitant with the onset of facilitation in cortical activity may be due to DC voltage components of the cortical electrogram during the violent metrazol discharge. Larger alkaline waves with metrazol discharge were related to overcompensated increase in blood flow.

6. The onset, intensity, duration and form of the metrazol discharge were not appreciably affected by variations in cortical pH between 7.0 and 7.5 induced by hyperventilation, CO₂ breathing, and by the injection of acid or alkaline solutions into the blood stream.

7. Changes in pH accompanying spontaneous cycles of prolonged epileptiform discharges alternating with periods of depressed cortical activity, following a single injection of metrazol, could all be explained upon the basis of the action of "acid" producing neuronal activity and its counteraction by increased blood flow. There was no apparent relationship between the level of pH and the occurrence of prolonged epileptiform discharge of this cyclic type following a moderate initial dose of metrazol.

8. Epileptiform after-discharge of one sigmoid gyrus resulting from nerve impulses conducted from the contralateral cortex subject to electrical stimulation was associated with changes in local cortical pH and blood flow of the discharging area similar in every way to those accompanying epileptiform discharge due to metrazol.

It is concluded that the facilitating action of metrazol, strychnine, and nerve impulses conducted from a distant area of cortex subjected to electrical stimulation is not primarily due to an increase in the local pH of the interstitial fluids of the cortex, because such an increase in pH occurring concomitantly with increased activity of cortical neurones is preceded by an increase in local blood flow. Increased pH seems to be the result of a vascular reaction to increased neuronal activity and does not seem to play an important rôle in the facilitation processes here studied.

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RELATION BETWEEN ELECTRICAL CHANGES DURING NERVE ACTIVITY AND CONCEN- TRATION OF CHOLINE ESTERASE*

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INTRODUCTION

ELECTRICAL changes during nerve activity occur in milliseconds or even in less than a millisecond. If chemical reactions are closely connected with these changes, the substances involved must appear and disappear with approximately the same rapidity. The difficulty of establishing exact time relations between electrical and chemical changes are therefore obvious. When, in 1933, Kibjakow and Dale and his associates suggested that acetylcholine (ACh) might be the transmitter substance of nerve impulses from pre- to postganglionic fibers (and later from motor nerves to striated muscles) this time factor became of primary importance. For the action of sympathetic and parasympathetic nerves on their effector organs the time factor did not have a comparable significance because cells in these organs react slowly.

No experimental data are yet available which establish the rate of ACh appearance during the period of nerve action. But some indications of the possible rate of removal of ACh from the sites of action have been obtained. ACh is inactivated by the specific enzyme choline esterase. Studies on the concentration and distribution of this enzyme have revealed that at motor end plates and ganglionic synapses as well as at synapses of the central nervous system considerable amounts of ACh can be split in milliseconds. These amounts if liberated would have stimulating action. The experiments, therefore, indicate that the removal of ACh can occur at a rate rapid enough for the assumption that ACh is involved in the transmitter process. The results have been recently reviewed (Nachmansohn, 1940a).

In this paper observations will be described which suggest a relation between electrical changes of nerve activity and the activity of choline esterase.

METHODS

The methods used were the same as described previously (Nachmansohn 1939). The tissues were ground with silicate in a physiological saline solution as homogeneously as possible. The saline solution was artificial sea water according to Pantin with the modification used previously. A fraction of the homogeneous suspension was taken for the determination of the enzyme activity with the manometric method of Barcroft-Warburg.

The determinations on the giant fiber were carried out with the Cartesian diver technique as described by Boell, Needham and Rogers (1939). Here too the tissue was ground with silicate and an aliquot part put into the divers.

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RESULTS

1. *Electromotive force of electric organs and concentration of choline esterase.* The high concentration of choline esterase at muscle end plates induced investigations on the enzyme activity in the electric organs of fishes because these organs are considered as an accumulation of muscle end plates. In view of the great power of the discharge, these organs are suitable for investigating the problem: are electrical phenomena connected with chemical reactions? There is no reason to regard the electrical activity of the elements from which the organ is formed, the electric plates or discs, as extraordinary when compared with a muscle of similar innervation. This has already been emphasized by the physiologists of the nineteenth century (Du Bois-Reymond, 1877, Burdon-Sanderson and Gotch, 1889). The essential difference by which electric organs are distinguished from other excitable structures is the arrangement of the electric plates in series like a Voltaic pile.¹ Hereby the effects of excitation increase in proportion to the number of elements in series.

The powerful electric organs (*Torpedo*, *Gymnotus*) can discharge 100–200 times per sec. All time relations of a discharge: duration, latency, refractory period, etc., are of the same order of magnitude as those observed in action potentials of ordinary nerves. Therefore, if ACh is involved, the same high concentration of choline esterase must be postulated as in the cases of motor end plates or synapses.

A high concentration of choline esterase was found in the electric organ of *Torpedo marmorata* (Marnay 1937, Nachmansohn and Lederer 1939, Nachmansohn 1940a). 100 g. organ split 200–300 g. ACh in 60 min. These figures appear remarkable for two reasons: (i), the enzyme concentration is of the same order of magnitude as that estimated for motor end plates of frog's sartorius; (ii), the organ has a concentration of a specific enzyme capable of splitting an amount of substrate 2–3 times the total weight of the organ in 60 min., in spite of the fact that electric organs contain about 92 per cent of water and only a little more than 2 per cent of protein. In the electric organ of a *Gymnotus electricus*² the enzyme concentration was also high although not as high as in *Torpedo marmorata*. In two species of *Raja undulata* which have a weak electric organ the concentration of choline esterase was relatively low (Nachmansohn 1940a).

¹ Volta himself detected the analogy between his pile and the electric organ and called the pile an "artificial electric organ" (quoted from Biedermann). There is however an important difference between the Voltaic pile and the electric organ. In the electric organ there is no resting current: if the two ends are connected there is no discharge as in the ordinary pile. The discharge is a voluntary act directed by the central nervous system. This fact induced Berzelius to assume that the discharge is connected with an organic chemical process. In his *Textbook of chemistry* (1817) he describes the electric organs under the title: "Electricity elicited by an organic chemical process" (Nachmansohn 1940b).

² The specimen examined died in October 1938 in the Institut Océanographique in Paris and a determination was made on the day following death in the Laboratoire de Physiologie Générale de la Sorbonne.

These observations strongly suggested a "cholinergic" nature of the nervous supply of the electric organ. This assumption was subsequently supported by evidence that ACh is liberated during stimulation and that injection of ACh produces a discharge. Eserine which alone is inactive increases manifold the discharge produced by ACh: 2.5 γ of ACh had a

Choline Esterase Distribution in the "Giant Fiber"
of the Squid (*Loligo pealii*).

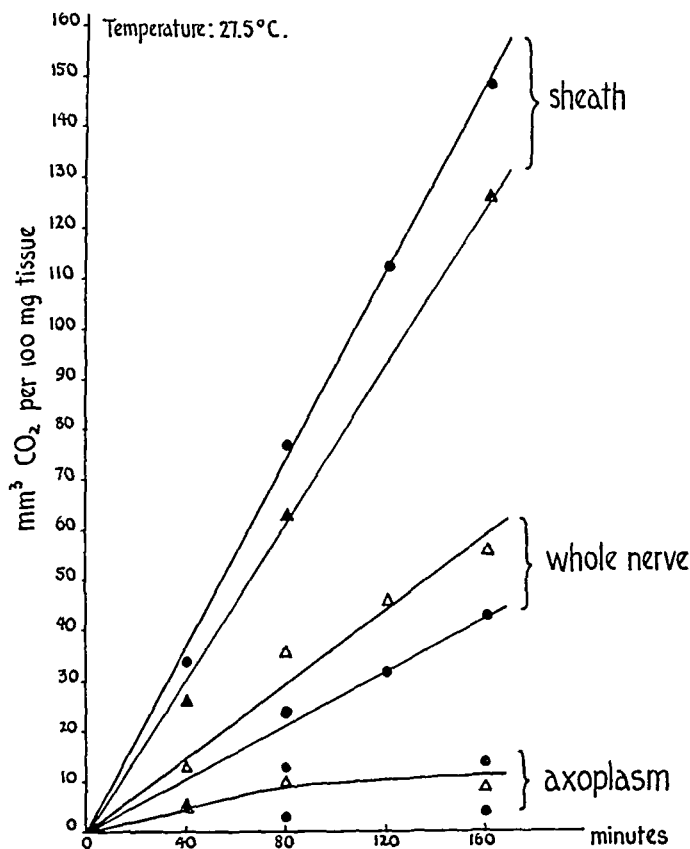


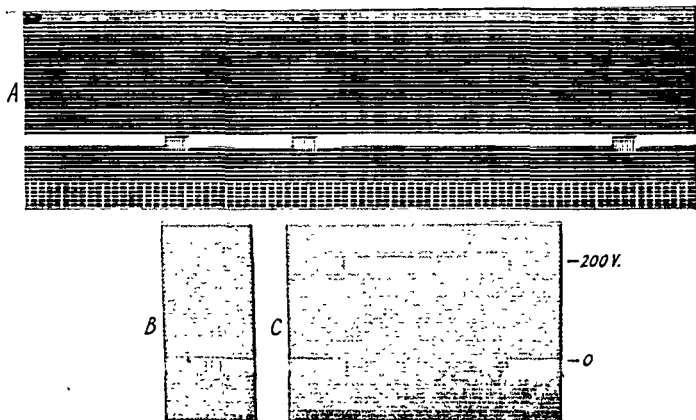
FIGURE 1

stronger effect on the eserized organ than 100 γ before eserization (Feldberg, Fessard and Nachmansohn, 1940).

If the discharge is closely connected with the appearance of ACh, it should be expected that a quantitative relationship exists between the electromotive force of the discharge, varying from one species to another, and the activity of the specific enzyme which has to remove the active substance during the refractory period. In view of the difference of the arrange-

ment of the electric discs and their number and frequency the best way to find out whether there is a parallelism between the potential differences and the activity of the enzyme appears to be to compare the E.M.F. per cm. and the number of discs per cm. with the concentration of the enzyme. For whatever may be the arrangement of the plates and their total number in series, and whatever may be the E.M.F. of a single plate, the E.M.F. per unit of tissue should be parallel to the concentration of the enzyme if liberation of ACh is directly connected with the potential difference.

The *Torpedo* has the greatest E.M.F. per cm. among the 3 species in-



A & B. Electrical Discharges of *Torpedo*. String at standard sensitivity. 100,000 ω in series with string. 1 ω in parallel. Peak voltages = A: 170, 155, 175 V. B: 225 V. C. Calibration. Deflection produced by 200 V. applied to large plate electrodes. (R. W. Amberson and D. J. Edwards. See footnote No. 4.)

vestigated. The maximum discharge is about 40–60 V. The extreme values indicated are 30 and 70–80 V. As the height of the columns in medium sized animals is about 1.5–2.5 cm. a cautious estimation would be 10–20 V. per cm., but it is probably nearer to 20 and may reach 30 V per cm. The number of plates in a column is about 400 which would mean an average of 150–250 plates per cm. The maximum discharge of *Gymnotus* according to the data in the literature is between 300 and 800 V. 400–600 V. is probably the most frequent value for the maximum discharge of a medium sized eel. That would be an average of about 6–8 V. per cm. for an 80 cm. organ of an eel of one meter length. But even assuming a larger limit, e.g. from 5–10 V. per cm., the E.M.F. per cm. is smaller than in *Torpedo marmorata*. According to the estimations of Ballowitz (1897) the number of discs in the main organ would

be about 60–80 per cm. in medium sized and 100–120 in small animals. In the "bundle of Sachs" the compartments are larger.³ In the Ray in which there are only about 15 discs per cm. the maximal discharge is only 1–3 V. and 0.5 V. per cm. according to Burdon-Sanderson and Gotch (4).

In Table I the concentration of choline esterase is compared with the estimations for the E.M.F. per cm. and number of plates per cm., in the three species examined: *Gymnotus electricus*, *Torpedo marmorata* and *Raja undulata*. The figures show a definite parallelism. The quantitative relationship may be even more precise if electrical, histological, and chemical studies could be made simultaneously on the same specimen.

In view of the great differences of the maximal discharge it is interesting to compare the maximal discharge with the total amount of ACh which can

Table 1. The relationship between the electromotive force of electric organs per cm. and the concentration of choline esterase.

Species	V per cm.	Plates per cm.	QCh.E.
<i>Raja undulata</i>	0.5	15	3–10
<i>Gymnotus electricus</i> (larger animals)	5–10	60–80	90–150
<i>Torpedo marmorata</i>	10–20	150–250	150–300

Table 2. The relationship between maximum discharge of electric organs, number of plates in series and amount of ACh which can be split by the organs in 1 sec.

Species	Maximum discharge in V	Number of plates in series	mg. ACh split by organ in 1 sec.
<i>Raja undulata</i>	1–3	60–80	0.5–1.0
<i>Torpedo marmorata</i>	30–60	400–500	50–100
<i>Gymnotus electricus</i>	300–800	5000–6000	500–1000

be split by an organ in a given period of time. Table II gives such a comparison for the same species which are compared in Table I. Here again there appears a definite parallelism. Such a parallelism is however less conclusive, for it is possible to imagine that in an organ the number of columns in parallel reaches very high values without a similar great number of plates in series. In that case the total amount of ACh which can be split in a given unit of time may be higher than the corresponding E.M.F.; only the amperage and power output would be higher.

We had the opportunity of examining 3 specimens of the large *Torpedo* (*T. occidentalis* Storer) which occasionally occurs in the water around Cape

³ These estimations are based on the assumption that E.M.F. per cm. and number of plates per cm. and enzyme concentration are rather uniform throughout the organ. More recent and detailed investigations however give a different picture (Coates, Cox and Nachmansohn).

Cod. The electric organs in this species are several times as large as in the *Torpedo marmorata*. There are no indications in the literature concerning the number of plates in series. The shock is described as being of a great power and only a little less strong than that of *Gymnotus* (Fritsch 1883, Rosenberg 1928). The E.M.F. of the discharge was recorded with a string galvanometer by W. R. Amberson and D. J. Edwards.⁴ Record A shows peak voltages of 170, 155 and 175 V., record B one of 225 V. Record C is a calibration record which shows the extent of galvanometer deflection caused by a voltage of 200 impressed upon the large metal plates which the authors used to press against the upper and lower surfaces of the animal. In most of the discharges

Table 3. Concentration of choline esterase in electric organs of *Torpedo occidentalis*, Storer

No.	Wt. of animal kg.	Length cm.	Width cm.	Ht. of columns cm.	Wt. of 2 elec. organs kg.	mg. wt. taken for determ. mg.	QCh.E.	TCh.E
1	13.5	—	—	2.8	2.5	12.0 43.5	284.0 290.0 271.0	7.0
2	39.2	123	90	7.6	9.0	22.0	79.0 73.0 80.5	7.0
3	9.0	80	52	3.0	2.0	27.0	226.0 227.0 211.0	4.0

TCh.E. = total amount of ACh in kg., which can be split by the 2 organs in 60 min.

the voltage was between 150 and 250 V. The authors feel that the peak voltages are actually higher than the values indicated by these records. The string galvanometer used was of a rather obsolete type and can hardly be expected to register the voltage accurately for such transient electrical discharges. In any case the voltages are no less than the records indicate.

The values for the concentration of choline esterase in the three specimens examined are high in the two smaller animals; in fact they are as high as in *T. marmorata* (Table III). This coincides with the observation that the discharge is much more powerful in the *T. occidentalis* than in the *T. marmorata*. As the electric organs in the former are several times as large as in the latter it should be expected that at the same enzyme concentration the discharge is more powerful in the large organs if E.M.F. and enzyme activity are correlated. In the giant animal the enzyme concentration is

⁴ Unpublished observations. The authors permitted us to mention their results and to reproduce some of their records in this paper. It is a great pleasure to express our gratitude for their kindness.

about one-third of that in the two smaller animals. This again is not surprising. The number of plates does not vary considerably from one individual to the other in a given species. Therefore as the height of the columns in the giant animal is 2-3 times as high as in the two others the concentration of choline esterase should be proportionally lower.

It has previously been shown (Feldberg, Fessard and Nachmansohn) that in the *T. marmorata*, the electric lobes and the nerves which come from these lobes and innervate the electric organs have a considerably higher enzyme concentration than the rest of the central nervous system. Therefore the distribution of choline esterase was determined in the central nervous system of a giant *Torpedo*. Table IV gives the data on some parts of the central nervous system. Although the electric lobes and the nerves innervating the electric organs have a relatively higher concentration than

Table 4. Concentration of choline esterase in some parts of the C.N.S. of the giant *Torpedo occidentalis*, Storer. Weight 34.5 kg. Length 132 cm.

Part of the C.N.S.	Wt. mg.	mg. wt. taken for determ.	QCh.E	TCh.E.
Cerebral hemisphere	370	=	1.4 1.3	5.3
Cerebellum		224.5	1.4 1.3	3.0
Hypophysis	52	=	0.98	0.5
Spinal cord		125.6	2.1	
Optical tract		26.0	2.0	
2 electric lobes	2308	37.0	4.2	97.0
Electric nerve near el. lobe		117.0	5.0	
Electric nerve near el. lobe		75.0	5.1	

the other parts of the central nervous system, the difference is not as great as that in *T. marmorata* and does not appear significant. The values do not lend support to the assumption that the nerves of the electric organs are distinguished by a higher concentration than ordinary nerves. Only the amount of nervous tissue is important and therefore the total amount of enzyme in the electric lobes as indicated by the TCh.E. is relatively high.

The enzyme concentration was also determined in specimens of two more species of Rays, *Raja erinacea* and *Raja eglanteria*. The figures obtained are given in Table V. The concentration is a little higher than that found in *Raja undulata*, but still of the same order of magnitude and low compared with that of the powerful electric organs.

II. *Localization of choline esterase in nerve cell.* The relationship between E.M.F. of the discharge in electric organs and the activity of choline esterase

leads to the question: is this relationship limited to nerve endings, either as the final event in the discharge of the electric organs or as part of the transmission process at synapses and motor end plates? Or are the potential differences which occur if nerve impulses travel down a nerve fiber alike and therefore also connected with ACh metabolism? Gasser and Erlanger (1939) in recently scrutinizing the problem came to the definite conclusion based on the electrical phenomena that conduction along fibers and across synapses is essentially the same process, as conceived previously by Eccles and Sherrington, and that the difference is only a quantitative one.

Liberation of ACh was generally considered as a process specifically limited to nerve endings and the quaternary base was therefore referred to as

Table 5. Concentration of choline esterase in electric organs of Ray.

No.	Species	Wt. animal gr.	Wt. el. organ taken for determin. mg.	QCh.E.
1	<i>Raja erinacea</i>	1000	71.0 97.0	17.5 18.3
2	<i>Raja erinacea</i>	1750	91.4 104.4	15.1 13.8
3	<i>Raja eglanteria</i>	1850	61.5 82.0	13.8 14.0
4	<i>Raja eglanteria</i>	2250	37.0 75.6	12.6 12.8
5	<i>Raja eglanteria</i>	1200	31.5 20.2	15.9 19.2

"synaptic transmitter." When, in 1938, Lorente de N6 found that ACh was liberated not only at synapses but also at fibers, his results were strongly criticized by Dale and his associates and a controversy arose over this problem (8, 14, 20, 21).

It has been emphasized on many occasions since 1938 that choline esterase is highly concentrated in all nerve fibers although it rises still more at regions where synapses and motor end plates exist. (Nachmansohn, 1938a and b, 1939). This was particularly obvious in non myelinated fibers, e.g. in the abdominal chain of lobsters where the QCh.E. values in the fibers were as high as 5.0-15.0, and rise at the points where the synapses are located to values of 18.0-30.0. There was a similar relationship in the distribution of the enzyme in the sympathetic chain of mammals. The conclusion was drawn from these observations that ACh metabolism in fibers and at synapses differs only quantitatively (25, 26).

Experiments carried out on the superior cervical ganglion of cats after section of preganglionic fibers suggest that the difference in enzyme con-

concentration between fiber and synapses may be connected with the difference of surface extension (Couteaux and Nachmansohn, 1939, 1940). The concentration of choline esterase in the ganglion decreases during the first ten days after section of preganglionic fibers by about 60 per cent and remains then stable for many weeks. As the decrease is parallel to the disappearance of the preganglionic fibers, it is reasonable to assume that the enzyme which disappeared was located *inside* the fibers, whereas the remaining enzyme was located *outside* the fibers. Under this assumption the figures obtained indicate that the enzyme concentration of the preganglionic fibers inside the ganglion is several times higher than the concentration in the same fibers before they enter the ganglion. These observations lead to the

Table 6. Distribution of choline esterase in the giant fiber of the squid (*Loligo paealii*) Temperature: 27.5°C.

No.	Kind of tissue	Mg. fresh tissue ground	Mg. fresh tissue per diver	Duration of experim. min.	Output of CO ₂ × 10 ³ mm. ³	QCh.E. (average)
1	Whole axon	2.6	0.078 0.078	185	141 118	0.438
	Sheath	0.55	0.017	185	69	1.130
	Axoplasm	2.6	0.078	185	12	0.040
2	Whole axon	3.3	0.123 0.123	160	53 69	0.150
	Sheath	2.4	0.09 0.09	160	135 115	0.420
	Axoplasm	5.5	0.20 0.20 0.20	160	8 28 18	0.027

conclusion that the strong increase of enzyme concentration in the preganglionic fibers towards their endings may be connected with the increase of surface occurring inside the ganglion by the extensive endarborization of the preganglionic fibers. They suggest that the enzyme may be higher concentrated at or near the surface and that the increase of surface explains the high concentration at synapses.

Bioelectric potentials are, it is generally believed, surface phenomena. Hodgkin and Huxley were recently able to prove this conception by measuring the potential difference between the inside and the outside of the giant axon of Squids (1939). The exact site of the membrane and the nature of the alteration which occurs there is not yet elucidated. Höber *et al.* (1939) studying the depolarizing effect of numerous organic and inorganic electrolytes and non-electrolytes recently discussed the different possibilities and aspects of the problem.

Table 7. Concentration of choline esterase in nervous tissue of *Squids*.

a. In fibers				b. In ganglia			
No.	Tissue	Wt. taken for det. mg.	QCh.E.	No.	Tissue	Wt. ¹ mg.	QCh.E.
1	Giant fiber	10.0	0.280	1	Stellate ganglion	35.4 ²	11.5
2	Giant fiber	8.5	0.214	2	Stellate ganglion	24.4	8.0
3	Giant fiber	12.5	0.210	3	Stellate ganglion	26.2	9.6
4	Giant fiber	11.5	0.170	4	Stellate ganglion	22.0	9.6
5	Whole trunk, containing giant fiber	21.6	0.444	5	Head ganglion	167.0	425.0 465.0
6	Whole trunk, containing giant fiber	22.6	0.750	6	Head ganglion	198.0	307.0 302.0
7	Whole trunk, containing giant fiber	29.6	0.544	7	Head ganglion	175.0	370.0 363.0
8	Finnerve	28.2	0.500	8	Head ganglion	167.0	270.0 280.0
9	Finnerve	27.2	0.485				
10	Finnerve	33.6	0.320				
11	Finnerve	23.6	0.416				
12	Finnerve	26.0	0.510				

¹ The weight indicates the total weight of the ganglion. The total amount was ground and an aliquot part taken for determinations.

² Two ganglia.

Obviously if choline esterase is concentrated at or near the surface of nerve fibers, as the experiments on the superior cervical ganglion suggest, it would strongly support the assumption that ACh is one of the substances involved in the electrochemical changes occurring at the surface during nerve activity.

Direct evidence is offered for this assumption with experiments on the giant fiber of the Squid.⁵ Determinations of choline esterase carried out with the diver technique as developed by Boell, Needham and Rogers, show that practically all the enzyme is localized in the sheath. The esterase activity of the axoplasm is negligible. Table VI gives the data obtained in two experiments. Experiment 2 was carried out with two and in the case of axoplasm with three parallel determinations. Figure 1 demonstrates the difference of the rate of hydrolysis in sheath, whole fiber and axoplasm respectively. 80-90 per cent of the sheath is connective tissue. The values for the sheath have

⁵ These experiments were made in collaboration with Dr. E. J. Boell of the Osborn Zoological Laboratory, Yale University. A preliminary report was given in *Science*, 1940, 92: 513-514.

therefore to be multiplied 5–10 times. But even then the values are minimum values: the enzyme concentration may be actually much higher than the activity per unit of tissue weight indicates, because it may be localized in a small fraction of the remaining volume.

The esterase concentration in the total giant fiber is intermediate between that for the sheath and that for the axoplasm. The values for the giant fiber with the ordinary Barcroft-Warburg method are about the same as those with the diver technique (see Table VII). Determinations on the total trunk containing the giant fiber reveal that the QCh.E. is higher than in the giant fiber itself. This is to be expected, for if the enzyme is concentrated at or near the surface, fibers with a smaller diameter should have a higher concentration of enzyme per unit of weight. As the fibers running in the same trunk have a smaller diameter than the giant fiber, the increase of QCh.E. is in agreement with that assumption. The trunk containing the giant fiber is composed of different types of fibers. Therefore the QCh.E. of the fin nerve which contains only motor nerve fibers has also been determined in order to compare functionally identical nerve fibers varying only in diameter. The values here too are definitely higher than in the giant fiber. The values in the stellate ganglion are about twenty times higher than in the giant fiber, the QCh.E. being about 8.0–11.0. This difference is of the same order of magnitude as that found between preganglionic fibers and the superior cervical ganglion of cats.

A particularly high value was found in the head ganglion exceeding all others so far, even those in the electric organ of *Torpedo*. It must be kept in mind however, that the protein content is about ten times higher in the head ganglion than in electric organs: it varied between 18 and 23 per cent

DISCUSSION

Two main facts result from the observations described: (i), the parallelism between E.M.F. and activity of choline esterase in electric organs indicates a quantitative relationship between potential differences of nerve activity and ACh metabolism. (ii) The enzyme is highly concentrated everywhere at or near the surface of the nerve cell suggesting that ACh metabolism occurs not only at synapses but everywhere at the surface of nerve cells, and is everywhere connected with the potential differences during nerve activity. The difference is only a quantitative one, correlated to the increase of surface at synapses. This again is in agreement with the observations on electrical changes from which Gasser and Erlanger came to the conclusion that there exists only a quantitative difference between conduction along fibers and across synapses.

Thus the battle cries of the last years: "electrical transmission" and "chemical transmission" lose their significance, because, in view of the results reported here, it is difficult to maintain the concept that ACh has a "transmitter" function specifically limited to nerve endings. The experiments suggest—in modification of the original theories of Loewi and Dale—

that ACh metabolism is intrinsically connected with the electrical changes of nerve activity which occur at the surface of nerve cells.

The objection may be raised that the conclusions are based on the presence of the enzyme and its concentration and not on determinations of the amounts of the ester actually metabolized. Choline esterase is a specific enzyme (Easson and Stedman 1937, Glick 1938, 1939). If a specific enzyme is highly concentrated in a cell, one may assume that the substrate is metabolized in such cells and that a relation exists between the concentration of the enzyme and the rate of the metabolism of its substrate. For such an assumption convincing evidence has been brought forward by the use of isotopes in the study of metabolism. As Schoenheimer and Rittenberg (1940) state in their review, the results with isotopes do not support the assumption that the tissue enzymes lie dormant during life, as some investigators believe: "The experiments indicate that all the actions for which specific enzymes and substrates exist in the animal are carried out continuously." This statement is particularly pertinent in view of the fact that the experiments with isotopes are carried out on intact animals.

At present it is difficult to go beyond the conclusion that ACh metabolism is closely connected with electrical changes occurring during nerve activity at the surface of neurons. A more precise formulation would require a knowledge of the time course of the appearance of ACh. This appearance may be the first, second or even the third step in a chain of events. It is hard to conceive that a biological process such as conduction or transmission of nerve impulses should be connected with a single chemical reaction.

SUMMARY

1. If in the electric organs of Ray, *Torpedo marmorata* and *Gymnotus electricus* voltage and number of plates per cm. are compared with the concentration of choline esterase, a close parallelism appears between E.M.F. and activity of the enzyme.

2. The enzyme concentration in the electric organ of the giant *Torpedo occidentalis* Storer is as high as in the electric organ of *T. marmorata*. This coincides with the observation that in the larger organs of the former species the E.M.F. is several times greater than in the small organs of the latter species.

3. No significant difference was found between enzyme concentration in electric lobes and nerves supplying the electric organs and that in other parts of the central nervous system in *T. occidentalis*.

4. Determinations of choline esterase carried out separately with sheath and axoplasm of the giant fiber of Squids (*Loligo paealii*) show that practically all the enzyme is localized in the sheath: the esterase activity of the axoplasm is negligible. This is considered as direct evidence for the previous assumption that the enzyme is concentrated everywhere at or near the surface of the nerve cell and that the rise of concentration at synapses is connected with the increase of surface.

5. In the head ganglion of the Squid a concentration of choline esterase has been found which is higher than in any other tissue examined thus far.

6. It is suggested that the potential differences observed during nerve activity may be closely connected with the metabolism of ACh everywhere at or near the surface of the nerve cell, the metabolism being only quantitatively more important at synapses.

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still gives rise to a small negative potential change at the regions of the neuro-muscular junctions. This local depolarization (the "end-plate potential" of Göpfert and Schaefer, 1938, and Eccles and O'Connor, 1939) (Fig. 1 and 2) can easily be distinguished from the ordinary muscle spike, (i) be-

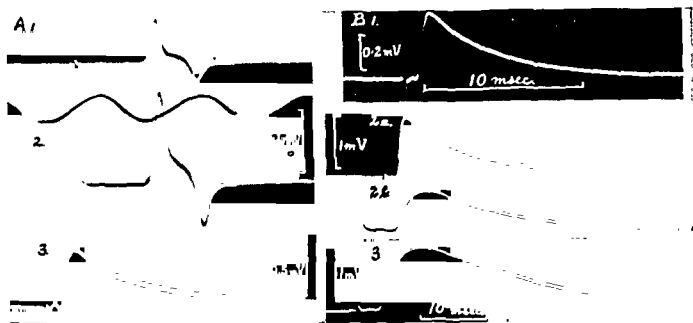


FIG. 1. "Endplate potentials" (e.p.p.'s) in curarized muscles. A1 and 2: Diphasic spike potentials of sartorius muscle at 25°C., due to direct stimulation; 1 half-maximal, 2 maximal; distance between leads 9.5 mm. 3: E.P.P., due to nerve stimulation. Time signal: 1 d.v. = 10 msec. Note: The small wave preceding the e.p.p. is due to the action potential of the intramuscular nerves (see also Fig. 4). B1: E.P.P. in cat's soleus muscle (at 38°C.). B2: E.P.P. in frog's sartorius; a, at 23°C.; b, at 10°C. B3: Another frog's sartorius, at 13.5°C.

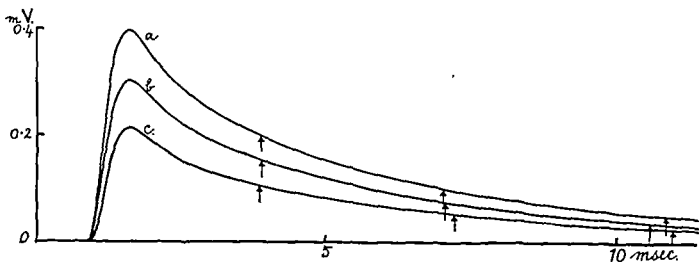


FIG. 2. Cat's soleus. E.p.p.'s with arrows showing points for decay to $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ of the summit height. (a) single nerve volley; (b) diminished response to second nerve volley at 24 msec. after first; (c) response to single volley diminished to nearly one half by further curarization. There is no significant difference in the three curves, and their successive half times progressively increase, being approximately 2.3, 3.3 and 3.7 msec.

cause it is not conducted along the fibres and (ii) because of its slower decay. It has been shown previously (Eccles and O'Connor, 1939; Katz and Kuffler, 1941) that the "endplate zones" can be located as the regions of minimum spike latency in the normal muscle, e.g. Fig. 3 gives evidence of several

such regions at which motor endings are accumulated. When the muscle is curarized, end-plate potentials (e.p.p.) are recorded at these regions and are often observed to decrement sharply at either side, becoming imperceptible a few millimetres away. In Fig. 4, for instance, there is a rapid diminution in size as the recording electrode is moved in either direction from the 11 mm. position; at the same time the e.p.p. waxes and wanes progressively more

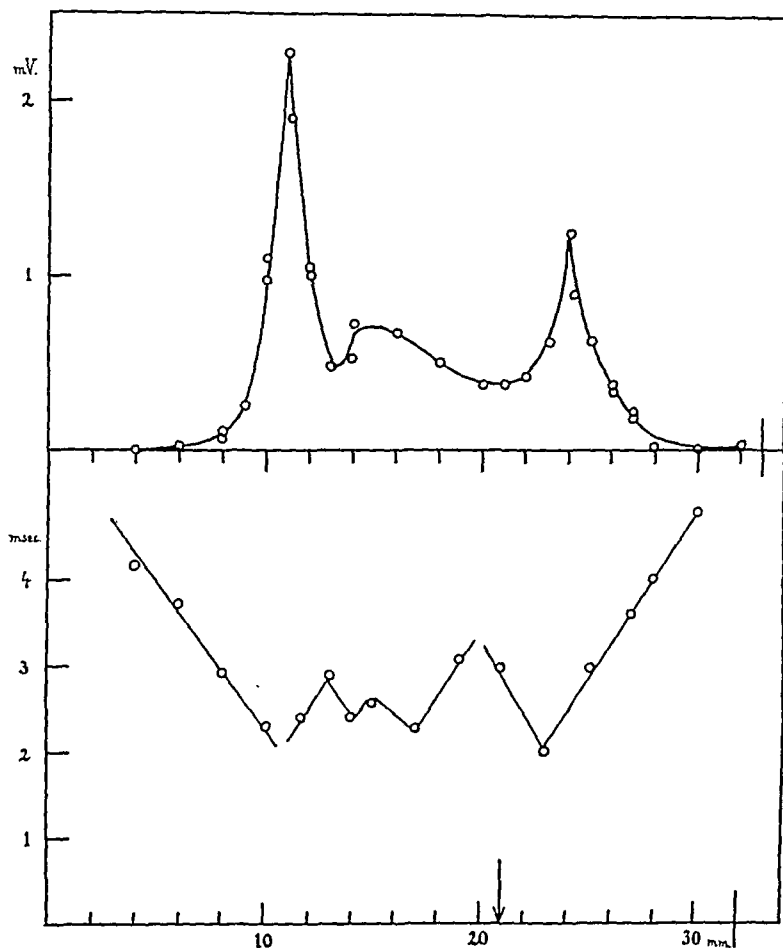


FIG. 3. Spatial distribution of e.p.p. in curarized frog's sartorius. Temp. 27.5°C. *Upper part:* completely curarized. Ordinates: Size of e.p.p. Abscissae: Position of earth lead, in mm. distance from pelvic end. Grid lead at pelvic end. Resting length 33 mm. *Lower part:* same muscle normally. Ordinates: Latency of muscle spike due to a nerve volley (shock-peak interval). Abscissae: as before. Resting length 32 mm. Position of nerve entry marked by arrow. Note the large e.p.p.'s at the regions of spike origin (at 11 mm. and 23-24 mm.).

slowly. In four preparations containing a sharply localized endplate focus the peak of the e.p.p. was found to decay to half in 0.5-0.9 mm. (fall to 1/e in 0.7 to 1.25 mm.).

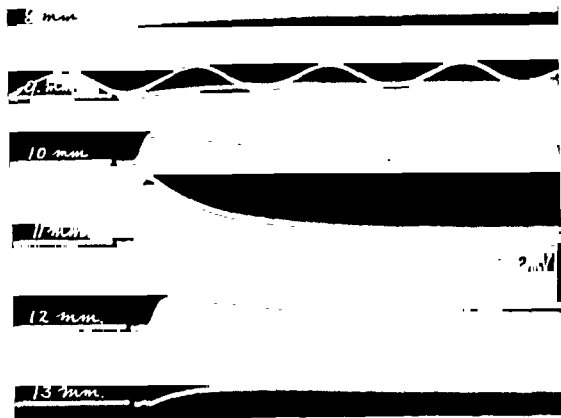


FIG. 4. Spatial spread of e.p.p. Same muscle as in Figs. 3, 5, 6. Records taken with grid lead at pelvic end and earth lead at a distance of 8–13 mm., as shown. Time signal: 1 d.v. = 10 msec. Note appearance of small nerve spike preceding the e.p.p., as electrode is moved towards nerve entry.

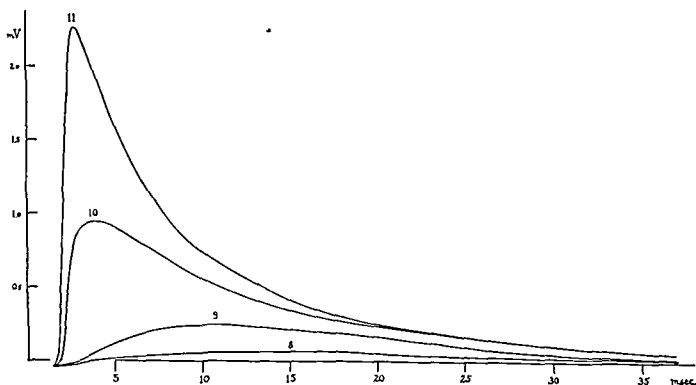


FIG. 5. Spatial decrement and accompanying change in time course of e.p.p. Records of Fig. 4 plotted and superimposed. Position of earth lead, successively from above: 11, 10, 9, 8 mm. distance from pelvic end. Abscissae: Time after nerve stimulus.

The characteristic spread of the e.p.p. in the vicinity of the motor nerve endings is of great interest. Its exponential decrement along the muscle and the associated slowing of its time course resembles the spread of electrotonic currents in nerve and muscle, or—more generally speaking—the spread of electric charge along a leaky capacitative cable (Cremer, 1909; Rushton,

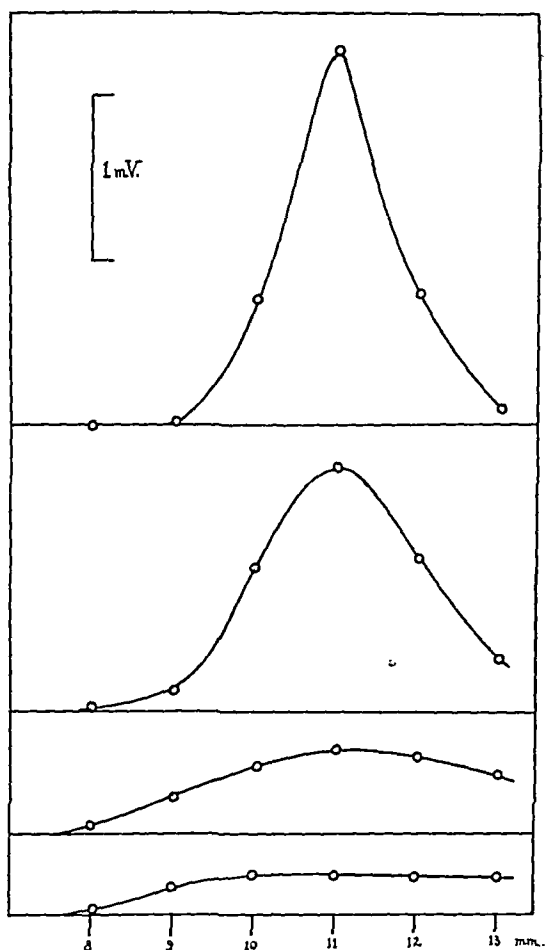


FIG. 6. Spatial distribution of e.p.p. (records of Fig. 4) plotted at successive time intervals. Time after nerve stimulus, successively from above: 2.8 msec.; 5.4 msec.; 13.3 msec.; 20 msec. Abscissae: Distance from pelvic end.

1934). An electric potential change set up at any given point of a nerve or muscle fibre causes the flow of local currents which spread a certain distance along the fibre, depending upon the resistance of the fibre and especially upon the transverse impedance of its surface membrane. In medullated nerve, the electrotonic potential spreads more extensively than in non-medullated nerve or muscle (the distances for $\frac{1}{2}$ decay are 2–3 mm. in frog's medullated nerve (Rushton, 1934; Bogue and Rosenberg, 1934;

Hodgkin, 1937), while only 0.5 mm. in crab's nerve (Hodgkin, 1938), and about 1 mm. in the frog's sartorius (Schaefer, Schölmerich and Haass, 1938). Since the surface membrane has a large distributed capacity (Cole and Curtis, 1936), the electrotonic potential does not rise instantly but only gradually, depending upon the rate of charge of the local membrane capacities. Thus, the greater the distance from the endplate focus, the slower is the rise of the recorded potential wave (Fig. 4 and 5) due to the delaying action of the intermediate membrane capacities. If the spatial distribution of the e.p.p. is plotted at successive intervals after the arrival of the nerve volley (Fig. 6), it is found that the e.p.p. continues to spread laterally even during its decay, in exact accordance with theory (Cremer, 1909).

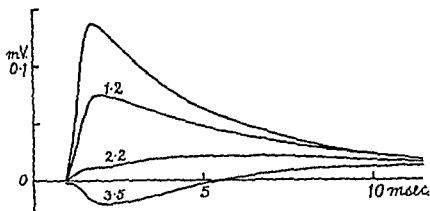


FIG. 7. Endplate potentials set up by a single nerve volley at various points along an innervated soleus strip of a cat. The largest potential is recorded at the endplate focus and the others at the indicated distances in mm. from the focus.

It might be argued that the spatial decrement of the e.p.p. is due to a scattered distribution of motor endings rather than to an extrinsic current spread from an adjacent focus of depolarization. In that case, however, the time course of the e.p.p. should be invariable and not exhibit the characteristic slowing associated with electrotonic spread. In the example of Fig. 4 a lateral scatter of motor endings more than 1 mm. away from the focal zone (at 11 mm.) cannot play an important part: at 9 mm. for instance, the e.p.p. reaches at its crest 12 per cent of the maximum peak (at 11 mm.), but its rate of rise is less than 2 per cent of that at the "focal region." It appears, therefore, that only a small fraction of the e.p.p. at 9 mm. can be due to the presence of scattered junctions at this point, and that at least 85 per cent of it is electrotonically transmitted from the focal zone 1-2 mm. away. Frequently, however, the endplates are not so sharply localized, and then, of course, the spatial potential gradient is made up in an unknown proportion by both factors: (i) the local density of endplates, and (ii) the electrotonic spread of depolarization from adjacent endplates.

With the soleus strip preparation of the cat the e.p.p. also becomes smaller and slower as the electrode is moved away from the endplate focus. At a distance of a few millimetres, however, the negative potential change is preceded by a positive initial wave (Fig. 7). This effect is due, not to an es-

sential difference between cat and frog muscle, but to the different conditions of electric recording in the two cases. As has been shown by Bishop (1937) and Bishop and Gilson (1929), an extensive fluid or tissue shunt, such as the inactive remainder of the cat's soleus muscle, distorts the local circuits in the vicinity of a depolarized region of the muscle surface in such a way as to make adjacent regions relatively positive to the more distal (cf. also Eccles and O'Connor 1939, p. 49). The effect can be imitated on the frog's sartorius by embedding it in a saline bath. This distortion precludes, of course, any accurate estimation of the spatial electrotonic decrement for the cat's soleus, an approximate value being half decay per millimetre.

B. SIZE AND TIME COURSE OF THE ENDPLATE POTENTIAL
IN RELATION TO THE MUSCLE SPIKE

It is clear from the preceding section that the time course of the e.p.p. depends to some extent upon the scatter of nerve endings within any particular junctional zone: for example, an endplate focus spread over 2 mm. will give a slightly slower e.p.p. than a focus restricted to 0.5–1 mm. of muscle length. In Table 1 the size and time course of the e.p.p. is compared with that of the normal propagating spike, both being recorded from the centre of a junctional region.

Table 1. End plate potential and normal muscle spike

(cf. Fig. 1 and 2) For typical records of spike potentials at the junctional region, see Eccles and O'Connor, 1939 (cat's soleus) and Katz and Kuffler, 1941 (frog's sartorius).

"Latency" (Maximum interval between arrival of nerve spike at junction and beginning of e.p.p. or spike respectively). msec.	Rising phase msec.	Fall to 1/e msec.	Size	
1.1–1.5	2.3	11	2–4 mV	e.p.p. (critically curarized). Frog's sartorius at 20° C.
1.1–1.5	1.6	1.5	40–50 mV	spike
0.6	0.8	4.5	5 p. c.	e.p.p. Cat's soleus.
0.7	0.6	0.6	100 p. c.	spike.

(i) *Latency*. The delay between the nerve stimulus and the start of the potential change at the endplate zone is but little altered by curarization. A large part of this delay is due to nerve conduction; as can be shown either by calculation or directly by high-amplification records which reveal the action potential of the intra-muscular nerves in the vicinity of the endplates (Fig. 1, 4). There remains a latency between the beginnings of this small nerve spike and of the muscle spike, or e.p.p. respectively, amounting

to 1.35 msec. (1.1–1.5 msec.) in the frog's sartorius at 20°C. and 0.6–0.7 msec. in the cat's soleus (Table 1). This delay hardly exceeds the duration of the nerve spike, and a fraction of it must again be due to conduction along the terminal nerve branches. It is affected by temperature changes to about the same extent as the duration or conduction rate of nerve or muscle impulses (see Table 3 below).

(ii) *Size.* The recorded size of e.p.p. and spike depends, though of course to a different extent, upon the distribution and local density of end-plates. The ratio of e.p.p. to spike must be greater the more strictly localized the end-plate zone, and greatest in a single isolated fibre. In 6 frog sartorii with relatively sharp endplate foci, the e.p.p. in critically curarized muscle (*i.e.* when abolition of spikes and associated twitches was just complete) reached 5–8 per cent of the normal spike (2–4 mV out of 30–50 mV); in 12 experiments on the cat the ratio was 3–6 per cent.

If the dose of curarine is increased beyond that required for complete paralysis, the e.p.p. becomes progressively smaller without, however, altering its time course (Fig. 2). Table 2 shows the relation between size of e.p.p. and curarine concentration.

Table 2. Relation between size of e.p.p. and dose of curarine

Frog's sartorius, at 16°C.					
Concentration of curarine, in $\mu\text{mol./l.}$	2	3	4	5	6
Size of e.p.p. in mV.	2.7*	1.2	0.75	0.5	0.34

* Spikes are still present in some fibres; peak of e.p.p. obtained by extrapolation of the rising phase, which has the same initial time course as with larger doses of curarine.

(iii) *Time course.* The e.p.p. is distinguished from the spike by a much slower decay. Its descending phase is approximately exponential, with a time constant of about 10 msec. at 20°C. for the frog's sartorius and 5 msec. for the cat's soleus. It has been pointed out by Schaefer and Haass (1939), (*cf.* also Katz, 1938) that the decay of the e.p.p. involves a time factor which is nearly the same as the time constant of the electrotonic potential (Schaefer, Schölmerich and Haass, 1938) or of the α -excitability of skeletal muscle (Rush-ton, 1930). In other words, the decline of the e.p.p. has about the same time course as that of an electric potential change in the muscle produced by an extrinsic current pulse. This relation is important because it suggests that the descending phase of the e.p.p. represents merely the passive decay of an electric change previously set up at the muscle membrane, in the absence of any further disturbing cause, just as would occur *after the withdrawal* of an applied cathodal current pulse (*cf.* Hill, 1936; Katz, 1939).

There are, however, both theoretical and experimental reasons (Cremer, 1909; Katz, 1939) for believing that the decay of the electrotonic potential in muscle does not follow a truly exponential time course, but is a function of the spatial and temporal distribution of

the applied current, *e.g.* the longer the current pulse, the slower should be the decline of the electrotonic potential. According to the electrotonic theory, successive half-times of decay of the e.p.p. should lengthen progressively. This is frequently observed (see *e.g.* Fig. 2), especially when the endplate focus is sharply localized. It may be that the strictly exponential decay found in many other preparations is really a distortion due to electrotonic potential spread from adjacent endplates. As a whole, therefore, the similarity of the time courses of the decay of e.p.p. and catelectrotonic potential, while suggestive of a similar mechanism, must be regarded with some caution.

C. THE EFFECT OF TEMPERATURE ON THE E.P.P.

In a series of experiments on the frog's sartorius, the effect of temperature on the time course of the e.p.p. was studied. In each experiment observations were made at two temperatures differing by 10 to 15°C., and ranging between 7 to 26°C., and were completed by returning to the original or an intermediate temperature. The results are illustrated in Fig. 8 and summa-

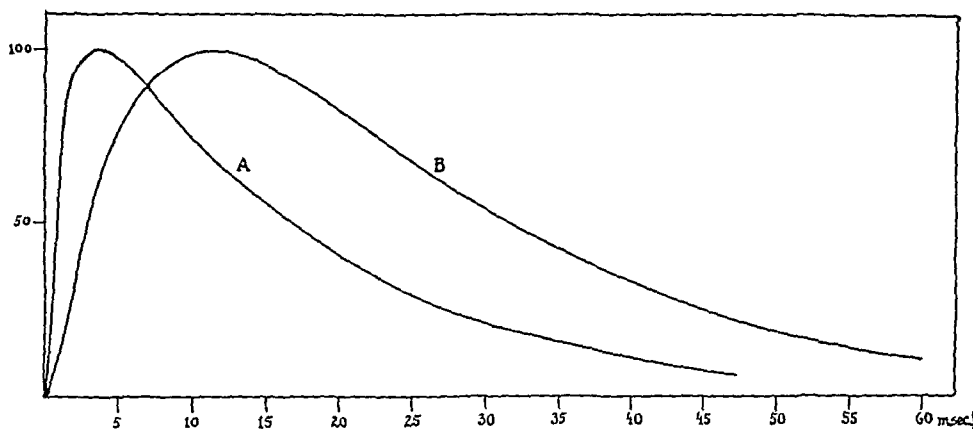


FIG. 8. A. E.p.p. at 19°C. B. E.p.p. of same muscle at 9°C. Scaled to same maximum. (The e.p.p. was 40% smaller at 9°C. than at 19°C.)

ized in Table 3. They reveal that the time course of decay of the e.p.p. is but little affected by temperature change ($Q_{10} = 1.3$), while the duration of the rising phase is lengthened nearly three times by a 10°C. lowering of temperature. It is clear that different processes underlie the building up and the decay of the e.p.p. It has been suggested above that the decay is mainly due to a passive dissipation of electrotonic charge along and across the muscle membrane. This view is supported by the low temperature coefficient (*cf.* Bogue and Rosenberg, 1934; Gasser, in Erlanger and Gasser, 1937). On the other hand, the building up of the e.p.p. presumably is a period during which the transmitter continues to act; it appears that the duration of this action is greatly increased by a fall of temperature (see Discussion).

Furthermore, at the lower temperature the e.p.p. is reduced in size, on the average by about 40 per cent.

D. INTERACTION BETWEEN ENDPLATE POTENTIAL AND "ANTIDROMIC" SPIKE

To obtain further information on the nature of the e.p.p., its interaction with a propagated muscle impulse was studied. A volley of muscle impulses

Table 3. Effect of temperature on e.p.p. in the frog.¹

	Time constant of decay of e.p.p. (fall to 1/e)	Time of rise of e.p.p.	Time constant of decay of "supernormal period of 2nd e.p.p." (see Section E)	Conduction velocity of muscle	"neuro-muscular latency" (Section B)
Mean value at 20° C.	11 msec.	2.3 msec.	39 msec.	1.6 m/sec.	1.35 msec.
Extreme values at 20° C.	7-16.5 msec. (25)	1.65-3.5 msec. (25)	30-50 msec. (12)	1.2-2 m/sec. (20)	1.1-1.5 msec. (14)
Temperature coefficient Q_{10} (ratio for 10° C. difference in same muscle), mean value.	<u>1.29</u>	<u>2.65</u> a) <u>2.7*</u> b) <u>2.6**</u>	<u>3.8</u>	<u>2.09</u>	<u>2.1</u>
Observed limits of Q_{10}	1.02-1.57 (5)	a) 2.1-3* (5) b) 2.15-3.5** (5)	2.6-5 (4)	1.9-2.6 (4)	1.95-2.2 (3)

¹ (Number of experiments shown in brackets.)

* Q_{10} for duration of rising phase.

** Q_{10} for reciprocal of maximum percentage rate of rise.

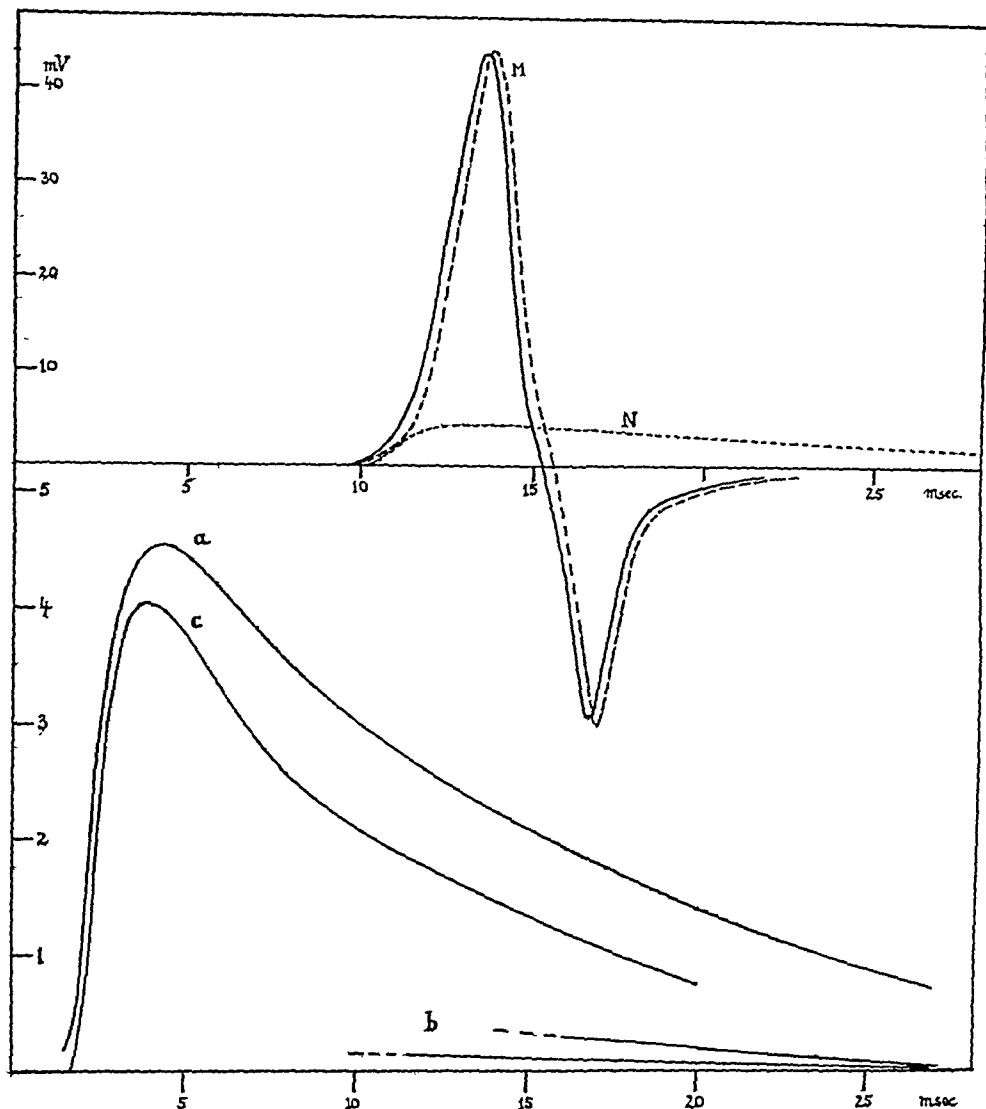


FIG. 9. Interaction between e.p.p. and muscle spike. Curarized frog's sartorius; 21°C. *Upper part*: Low amplification. Broken lines: *N*, e.p.p. due to nerve volley; *M*, diphasic spike due to direct stimulus. Full line: potential due to combined stimulation *NM*. Note, slight speeding of spike, no addition to spike peak. *Lower part*: High amplification. *a*, normal e.p.p. *b* and *c*, additions of e.p.p.'s during and after spike, obtained by curve subtraction ($MN - M$). *b*, remainder of e.p.p. set up simultaneously with spike (lower line), and 2 msec. before (upper line). *c*, e.p.p. set up 24 msec. after spike.

(henceforth called the "antidromic" volley) was set up in the frog's sartorius by stimulation at its nerve-free pelvic end and was superimposed at various time intervals on an e.p.p. (Fig. 9-12). Accurate investigation is only possible with large e.p.p.'s such as recorded from a sharply localized endplate focus in a state of critical curarization.

To summarize the main findings, (i) the antidromic impulse was slightly speeded up while travelling through the partially depolarized junctional region (Fig. 9, 10), (ii) the e.p.p. summed perfectly with the initial "foot" of the muscle spike, but added little (Fig. 11 B) or nothing (Fig. 10; 11 A) to its peak and descending phase.

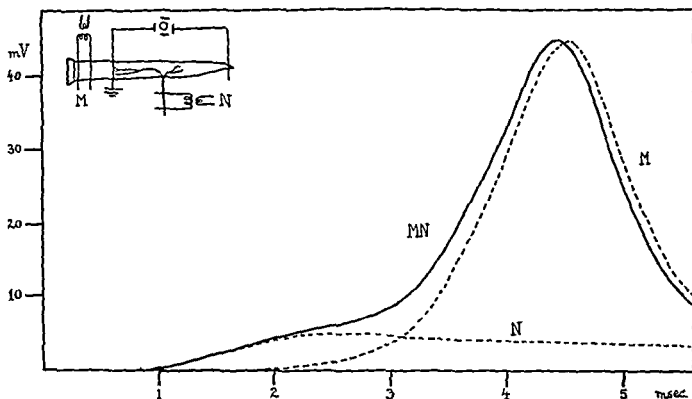


FIG. 10. E.p.p. and muscle spike. Frog's sartorius, at 22°C. Inset: preparation with stimulating (*N* nerve, *M* muscle) and recording leads. Broken lines: *N*, e.p.p. set up by nerve stimulus; *M*, antidromic spike set up by direct stimulus. Full line: potential change due to combined *MN* stimulus.

(i) *The effect of the e.p.p. on the propagation of the muscle impulse.* If the e.p.p. was set up simultaneously with, or a few msec. before, the arrival of the muscle impulse, it facilitated the propagation of the spike. The magnitude of this effect varied from experiment to experiment, depending no doubt upon variations in the local excitability of the muscle and in the degree of curarization. The maximum speeding was observed when the action potential was recorded at the tibial end, after it had travelled through all the "endplate zones" of the muscle, i.e. past two or three successive junctions in each individual fibre (cf. Katz and Kuffler, 1941). The greatest speeding amounted to 0.35 msec., equivalent to the conduction time for about 0.7 mm. of fibre length. It will be seen below that, when above a certain critical level, an e.p.p. gives rise to a propagated action potential; below this level, it exerts a subthreshold excitatory action on the muscle fibre, and this must be responsible for the speeding of the muscle impulse.

Though local depolarizations normally produce excitatory effects on nerve and muscle fibres, it has frequently been shown (e.g. Werigo, 1901; Blair and Erlanger, 1933, Fig. 11; Bugnard and Hill 1935; Katz 1937) that they exert inhibitory or even blocking actions, (i) when maintained at a high level for a long duration, (ii) when applied during a refractory

state. It is easy to understand, therefore, that the muscle impulse, while helped along by an ordinary e.p.p., is delayed or blocked by a cumulative and prolonged depolarization (Feng, 1937): the depression produced by an early second nerve volley (Eccles and O'Connor, 1939a; Eccles, Katz and Kuffler, 1941), and the well-known Wedenski-inhibition in normal and especially in eserinizied muscle accompanied by a piling up of repetitive endplate potentials (Feng, 1939) are readily explained on this basis.

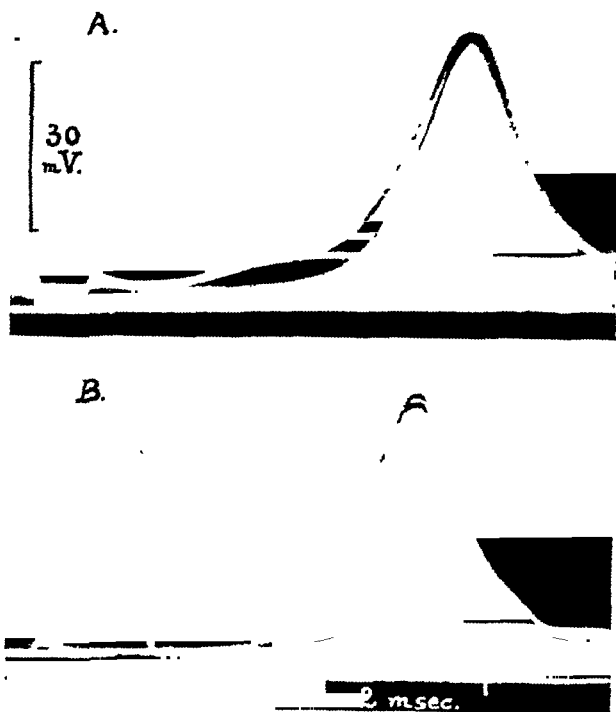


FIG. 11. Superimposed records of (i) antidromic spike, (ii) e.p.p., and (iii) combined potential change. Completely curarized frog's sartorius, at 22°C. A: Same as in Fig. 10. B: E.p.p. set up during spike. The e.p.p. gives a slight addition which rapidly disappears.

(ii) *The breakdown of the e.p.p. during the muscle spike.* As shown in Fig. 11 an e.p.p., if superimposed on an antidromic muscle spike, does not add its full height to that of the propagated potential wave. In fact, after an initial summation of the e.p.p. and the spike "foot" (Fig. 10), the e.p.p. seems to disappear completely, since neither the summit nor the descending phase of the action potential shows any addition or any alteration apart from a slight speeding. If allowance is made for this slight time shift of the later parts of the spike, the approximate shape of the superimposed e.p.p. can be obtained by a simple curve subtraction (Fig. 12). It appears that the e.p.p., after helping the antidromic impulse in its approach to the junctional region, is obliterated within 2 msec. during the ensuing spike process.

The recent work of Hodgkin (1937, 1938) and Cole and Curtis (1939) has shown (i) that the "foot" of the propagating action potential is identical with a subliminal depolarization of the axon membrane, and is caused by an

electrotonic spread of local action currents; and (ii) that at a certain critical potential level, "membrane excitation" occurs associated with a sudden breakdown of its transverse resistance. This change gradually dies away, as the membrane passes out of its refractory period. The present observa-

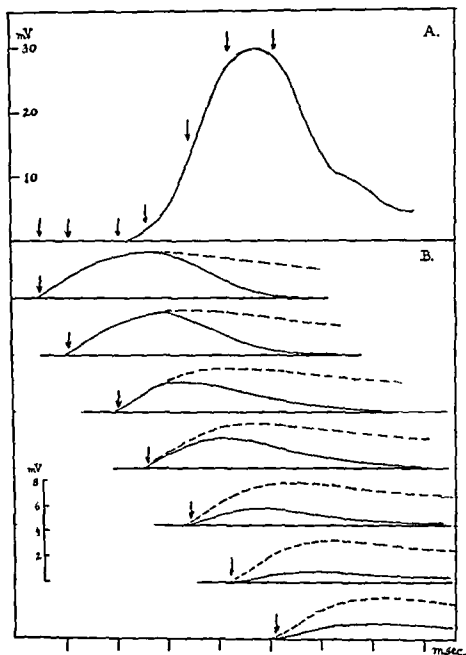


FIG. 12. Change of e.p.p. during passage of spike. Frog's sartorius at 22°C. completely curarized. A. Antidromic spike. B. Full lines: E.p.p.'s superimposed at various time intervals on the spike (see corresponding arrows in A). Broken lines: Control curves of e.p.p.'s alone.

tions are in good agreement with this concept. During the "foot" of the spike the two potential waves (e.p.p. and action potential) sum as two catelectrotonic potentials would do; as a consequence the critical threshold level is reached sooner and propagation speeded up. During the subsequent state of activity, the physical properties of the membrane undergo a sudden change which affects the size and time course of any superimposed electrotonic potential. As a result of the transverse resistance breakdown (Cole

and Curtis, 1939) the electric time constant of the membrane (i.e. the product RC of its transverse resistance and capacity) is greatly reduced, and any applied electric charge quickly leaks away. It follows that the decaying remainder of an e.p.p. rapidly collapses (Fig. 12). Further, a nerve volley arriving during this state of the membrane can only build up a much diminished e.p.p. (about 30 per cent, Fig. 12), just as in a non-medullated nerve fibre a stimulus, if superimposed on a propagating spike, produces a greatly diminished electrotonic potential (Hodgkin, 1938, p. 107).

If the nerve volley arrives at the myoneural region at progressive intervals *after* the antidromic impulse, the e.p.p. gradually recovers its normal size and duration. Even after 24 msec., however, the e.p.p. is depressed by 13 per cent below normal (Fig. 9). There is a possibility that this late depression might be only apparent, and really due to a slight movement of the muscle relative to the recording lead, but this has been checked by recording successively at a little distance to either side of the endplate focus.

It was observed in some cases that the antidromic spike left behind it a period of super-normal excitability during which neuro-muscular transmission was partially restored: in such preparations, for example, an e.p.p. set up 8 msec. after the antidromic spike was capable, in spite of its subnormal size, of eliciting propagated action potentials in some fibres.

The observed interactions between muscle spike and e.p.p. permit a number of inferences: they confirm the view that the e.p.p. is a subthreshold alteration of electric charge at the muscle membrane, and also provide evidence for an electric propagation of the muscle impulse in accordance with the Hermann-Lille hypothesis and Hodgkin's and Cole's recent observations on nerve.

Moreover, the fact that the descending part of the e.p.p. does not "survive" the passage of a muscle spike indicates that the depolarizing agent responsible for the *production* of the e.p.p. has ceased to act long before the latter has disappeared. If this were not so, the e.p.p. should be only temporarily depressed during the spike and afterwards built up again to its normal amplitude. This is indeed observed in the eseriniz preparation (Eccles, Katz and Kuffler, 1941), where the depolarizing agent persists for a long period and so rebuilds the e.p.p. after the passage of the spike; in the same way, a catelectrotonic potential in a nerve axon recovers after the discharge of an action potential, provided the applied current is maintained (Hodgkin and Rushton, unpublished). In the curarized preparation, however, the remainder of the e.p.p. is permanently reduced to less than 20 per cent, or completely abolished, by an intervening spike (the small addition in Fig. 9 is probably due to uncorrected time shift of the *MN* curve). Thus it seems that most of the declining phase of the e.p.p. is a passive decay of a negative membrane charge after the depolarizing agent has ceased to act. The earlier suggestion, therefore, that the decline of the e.p.p. follows the time course of a passively decaying electrotonic potential (see p. 370) is confirmed.

This property of the e.p.p. differs markedly from that of a "negative

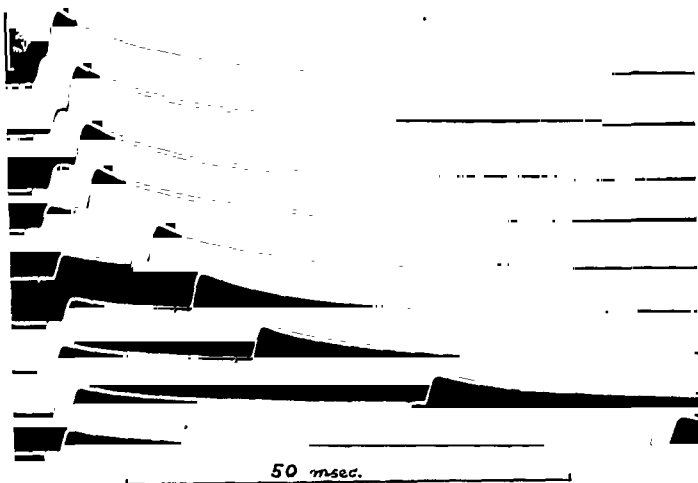


FIG. 13. Summation of e.p.p.'s with two successive nerve volleys. Frog's sartorius, at 25°C.

after-potential" which, as Gasser (in Gasser and Erlanger, 1937) has shown, sums with a subsequent spike and, to some extent, piles up during tetanic stimulation. This difference is of interest since there was previously some doubt (e.g. Katz, 1939) whether the e.p.p.—and its associated excitatory effect—is analogous to a "negative after-potential" extending to the myoneural junction and associated with a super-normal phase of transmission, or—as supported by the present findings—to a "local potential" (Hill 1936) in the muscle fibres which declines slowly because of the long time constant of the muscle membrane.

E. THE MECHANISM OF NEURO-MUSCULAR FACILITATION

It has been shown in section *D* that the e.p.p. acts like a subliminal catelectrotonic potential of the muscle fibres and can add its excitatory effect to that of the subliminal current spreading in front of an advancing action potential wave. From this it may be inferred (i) that two e.p.p.'s set up by successive nerve volleys will sum as successive catelectrotonic potentials would do (see Schaefer and Haass, 1939), and (ii) that the e.p.p., if above a certain threshold level, must itself be capable of eliciting propagated action potentials. Furthermore, in a critical state of curare-block, neuro-muscular transmission should be restored by the summation (a) of successive e.p.p.'s

or (b) of an e.p.p. and a subliminal electric current pulse applied to the myoneural region, as was found by Schaefer and Haass (1939) and Katz (1938).

A. *Frog's muscle*. Summation of two e.p.p.'s in the frog's sartorius is illustrated in Fig. 13 and 14. These figures reveal an unexpected fact, namely that the e.p.p. produced by the second nerve volley is *greater* than the first

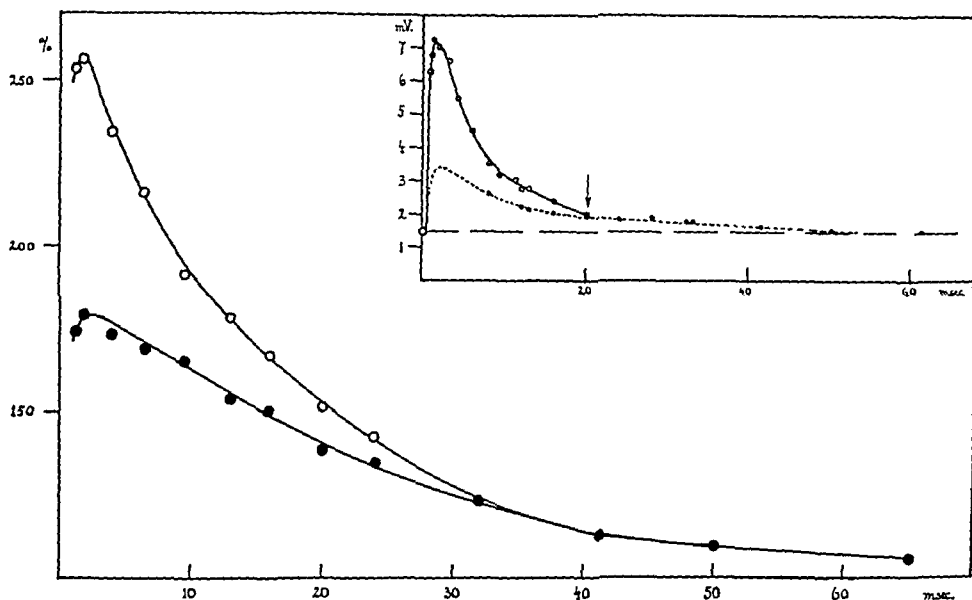


FIG. 14. Summation of e.p.p.'s with 2 nerve volleys (cf. records in Fig. 16B). Frog's sartorius at 25°C. completely curarized (6 μ mol. curarine). Abscissae: Time interval between nerve volleys. Ordinates: Size of e.p.p. in p.c. of single e.p.p. Hollow circles: total height. Full circles: height of added second e.p.p. INSET (cf. FIG. 16A): Facilitation in partially curarized muscle (3 μ mol curarine). Ordinates: Peak potentials. Full line (hollow circles): total potential height (spike + e.p.p.). Arrow indicates threshold level of e.p.p. (2 mV) at which spikes are initiated. Broken line (full circles): Height of summed e.p.p.'s. With intervals less than 8 msec., the e.p.p. was obscured by spikes. Broken curve shows height which summed e.p.p.'s would reach in absence of spikes (as with 6 μ mol curarine).

(Schaefer and Haass, 1939; Feng, 1940), though its time course is unchanged. The summed potential may reach 280 per cent, and the "supernormal" second e.p.p. 200 per cent of the height of the single e.p.p. (see Table 4). The

Table 4. Supernormal size of second e.p.p. in the frog's sartorius

12 experiments. at 23.5°C. (17.5–29°C.)	Size, in per cent of single e.p.p.	
	total height. 264 (240–296)	height of added 2nd e.p.p. 188 (171–206)

Time factor k'' of decay of "supernormal period" (k' = average time for fall to 1/e):
At 20°C., k'' = 40 msec.

Observed limits for k'' :

8.5 msec., at 31.5°C.

190 msec., at 8.6°C.

Q_{10} = 3.8 (2.6–5, in 5 experiments).

"supernormal" effect dies out within about 0.1 sec. at 20 C. (Fig. 15). Its rate of decay has a remarkably high temperature coefficient, being slowed about 4 times by a 10 C. temperature reduction.

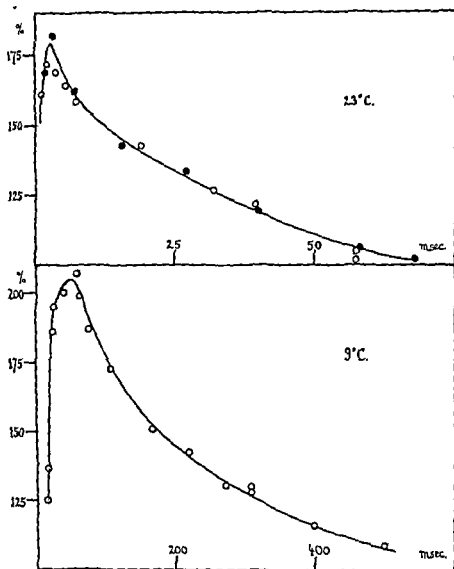


FIG. 15. "Supernormal period" of second e.p.p. Completely curarized frog's sartorius. Ordinates: Size of second e.p.p. in p.c. of the first. Abscissae: Time interval between two nerve volleys. Upper part: 23°C. 2 series of observations, before (hollow circles) and after (full circles) low temperature series. Lower part: 9°C.

With a smaller dose of curarine, summation of two e.p.p.'s may give rise to propagated spikes and mechanical twitches, i.e. to "neuro-muscular facilitation" (*cf.* Adrian and Lucas, 1912; Bremer, 1927). In the case of Fig. 16, a single nerve volley produces a subthreshold e.p.p. on which a second volley adds its e.p.p. after various intervals. Whenever the summed potential wave exceeds a level of 2 mV, propagated spike potentials appear. The observed size of the critical potential level at which muscle spikes are initiated depends, of course, upon the distribution of motor nerve endings: in preparations with a sharp endplate focus up to 4 mV (8 per cent of the spike) has been found (*cf.* section B). At the critical volley interval (Fig. 16) the spike is seen to take off at the peak of the e.p.p. With shorter intervals, the summed e.p.p. progressively exceeds the threshold level; the spike, there-

fore, becomes larger, and takes off during the rising phase of the second e.p.p. The critical threshold is illustrated even better on the strip preparation of the cat (Fig. 18), where the spike is simple and synchronous (Eccles and O'Connor, 1939).

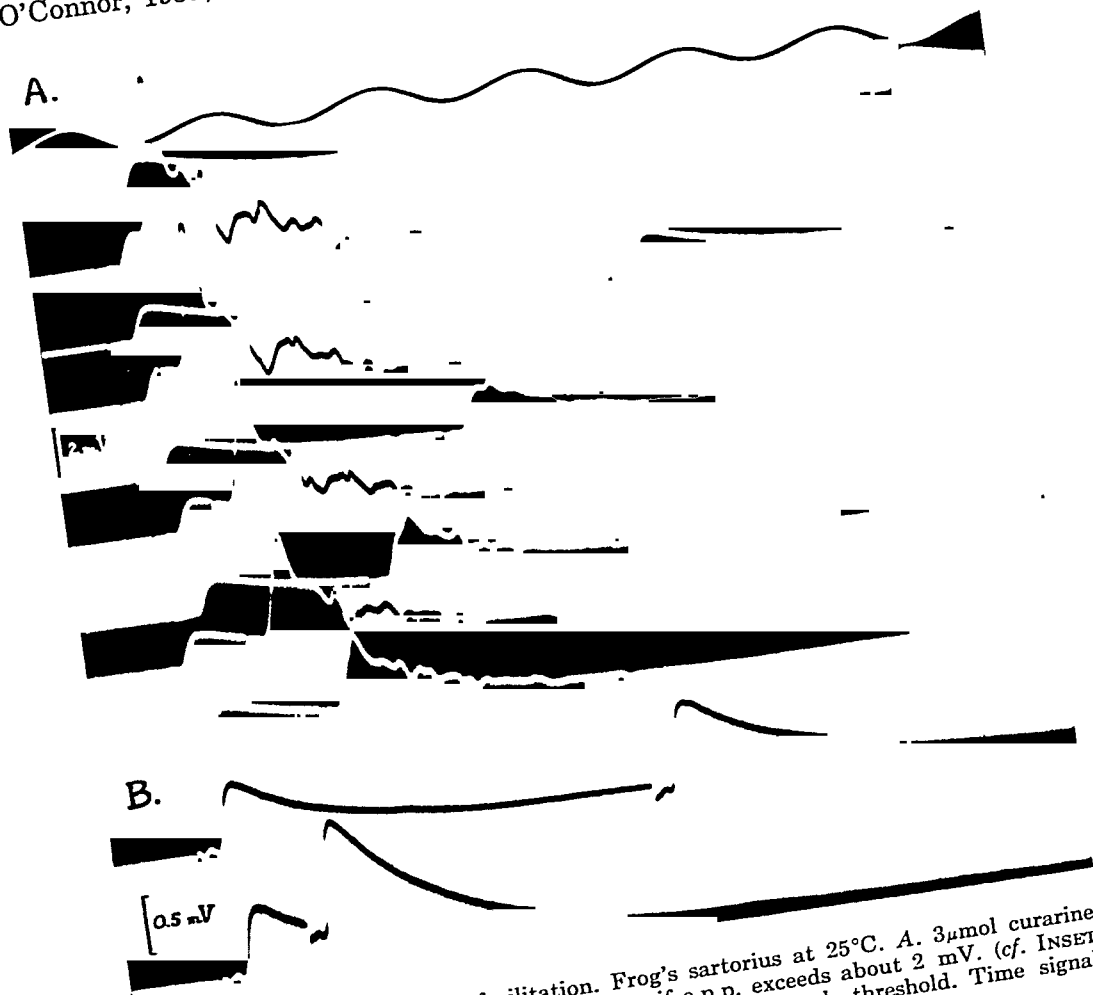


FIG. 16. Neuro-muscular facilitation. Frog's sartorius at 25°C. A. 3 μ mol curarine. Appearance of spikes with two nerve volleys, if e.p.p. exceeds about 2 mV. (cf. INSET, FIG. 14). B. 6 μ mol curarine. Summed e.p.p.'s do not reach threshold. Time signal: 1 d.v. = 10 msec.

The irregular polyphasic shape of the spikes in the partially curarized sartorius (Fig. 16) is due to their scattered points of origin along about 2.5 cm. of muscle (Katz and Kuffler, 1941). In the normal preparation the late spikes are not recorded because they collide with impulses arising, in the same muscle fibres, from the endplate nearest to the recording lead (Katz and Kuffler, 1941). Furthermore, in a critical stage of curarization, single fibre impulses sometimes arise quite late—on the falling phase of the e.p.p. Such long latencies might be due to a local response (Hodgkin, 1938) which at threshold level may linger on for a few milliseconds before giving rise to a propagated spike.

The magnitude and time course of neuro-muscular facilitation in the frog depend upon two factors: (i) upon the size and time course of decay of the first e.p.p., on the declining remainder of which the second e.p.p. is added, (ii) upon the supernormal size of the second e.p.p. This supernormal effect outlasts the single e.p.p. and, therefore, a remainder of neuromuscular facilitation can persist beyond the period during which there is actual summation of the two e.p.p.'s.

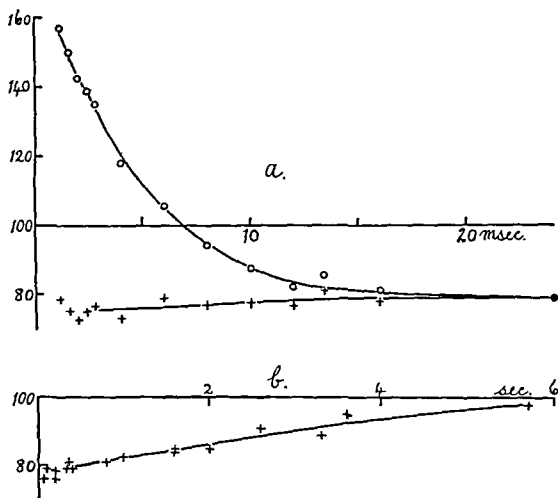


FIG. 17. Cat's soleus. Plotting as in Fig. 14 of e.p.p.'s set up by a second nerve volley (a) Circles: total size. Crosses: size of added second e.p.p. (b) shows recovery of added second e.p.p. at longer volley intervals.

At about 30°C., the e.p.p. set up by the first nerve volley and the supernormal phase of the second e.p.p. subside at about the same speed, while at 10°C., owing to the different temperature coefficients of the two processes, the supernormal effect decays about 9 times more slowly than the first e.p.p. Hence, as the temperature is lowered, the duration of neuro-muscular facilitation progressively exceeds that of the single e.p.p.

Thus, the earlier suggestion (Katz, 1939), viz, that the gradual subsidence of neuro-muscular facilitation is due to the electrotonic decay of a local depolarization of the muscle fibres is only partly confirmed; the "supernormal" size of the second e.p.p. is an additional factor at least in frog's muscle. The nature of this supernormal effect cannot at present be explained, but it is interesting to note that the effect is greatly reduced, or even abolished, by eserine, as will be reported later.

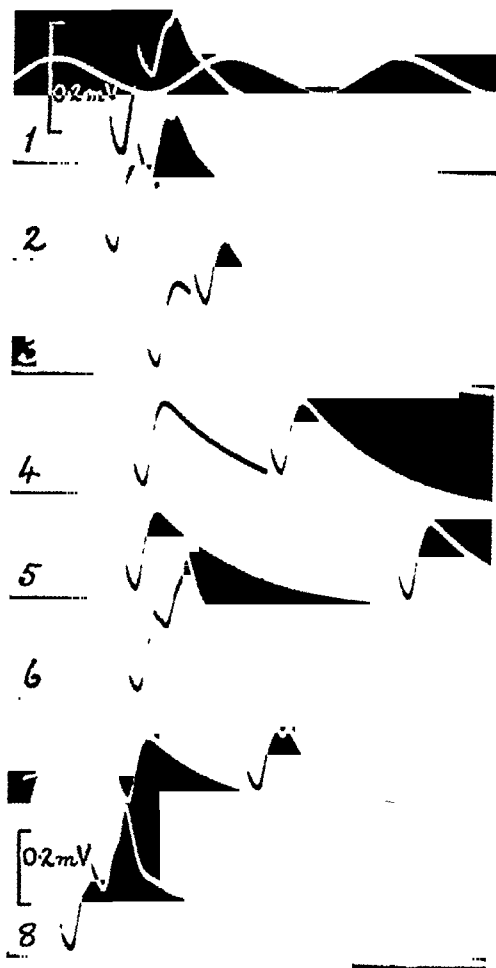


Fig. 18. *Cat's soleus*. E.p.p.'s set up by 2 nerve volleys at indicated intervals apart. 1 and 2: summed e.p.p. reaches 0.26 mV and a small muscle spike (possibly in a single fibre) arises at the summit. 3: A slightly lower e.p.p. (0.25 mV) fails to set up a spike. 6, 7 and 8: less deep curarization (single e.p.p. 0.205 mV; 0.165 mV for earlier series) and correspondingly the small spike is set up even at 8 msec. interval (summed potential 0.24 mV). The larger e.p.p. at shorter intervals (at least 0.32 mV in 6 and 8) sets up impulses in additional fibres as shown by the larger spike. Thus 0.24 to 0.25 mV is the critical e.p.p. for setting up spike. Time: 1 d.v. = 10 msec. Potential scales shown for 1st and 2nd series. Apparent positivity at end of records due to amplifier distortions.

In some experiments there was evidence of a protracted subnormal period, with intervals greater than 0.15 sec., though the depression did not exceed 5 per cent.

B. *Cat's muscle*. In soleus (also peroneus tertius and tibialis anticus) the second e.p.p. is always *diminished* by about 80 per cent (70–95 per cent). Figure 17 shows that this depression persists for a few seconds—full recovery requiring 2.5 to 10 sec. As a result of this depression, neuro-muscular facilitation in the cat is much less conspicuous than in the frog. The summed e.p.p.'s reach a maximum height of only about 170 p.c. of the single e.p.p., and the period of facilitation is terminated at an interval of about 8 msec. (6–20 msec., cf. Fig. 17 and 18). Beyond this interval, the summed potential is smaller than the first e.p.p. In partially curarized muscle, therefore, the spike response to a second nerve volley is diminished at such intervals (cf. Brown, 1938).

DISCUSSION

The nature of the e.p.p. The present experiments have shown that a nerve impulse arriving at a curarized myoneural junction sets up a local depolarization in the muscle fibre (the "endplate potential") which acts in all respects like the local potential produced by a sub-threshold electric stimulus. The evidence relating to this may be summarized as follows.

(1) The e.p.p. spreads along the muscle with a decrement of about 50 p.c. per mm., and with progressive slowing of its time course (Section A).

(2) The time factor involved in the decay of the e.p.p. (Section B) is of the same order as that of the catelectrotonic potential (Schaefer and Haass, 1939) or the α -excitability of muscle (Rushton, 1930). This time factor has a low temperature coefficient ($Q_{10}=1.29$) as expected for a passive electrotonic decay (Section C).

(3) The e.p.p. sums with the initial "foot" (i.e. the extrinsic passive phase) of an approaching muscle impulse just as a catelectrotonic potential would do, and the impulse propagation is speeded up (Section D).

(4) During the "active phase" of the muscle spike (cf. Hodgkin 1937, 1938), the e.p.p. resembles a catelectrotonic potential in that it is built up to a much smaller potential and its decay is much more rapid (Section D).

(5) If the e.p.p. exceeds a certain critical level, a muscle impulse is initiated (Section E). Facilitation of neuro-muscular transmission occurs when summation of successive e.p.p.s gives a sufficiently high level (Section E).

The transmitter. The present work does not indicate how the endplate potential is produced. It might be due to the depolarizing influence of local action currents, or to a transient effect on the muscle membrane by a chemical transmitter such as acetylcholine. Our present knowledge concerning the mode of action of curare appears to favour the acetylcholine theory. Curare greatly diminishes the stimulating action of nicotine (Langley, 1909) and of acetylcholine on skeletal muscle (Brown, Dale and Feldberg, 1936), and this parallels its powerful action (Section A) in diminishing the endplate potential, i.e. the depolarizing effect of the transmitter. Furthermore, Lapicque's evidence that curare alters the electric excitability of the muscle can now be considered as disproved (see Rushton, 1930; Schaefer, Schölmerich and Haass, 1938). However, it may well be that curare has various other actions at the myoneural junction (cf. its action on nerve: Fromherz, 1933; Fessard, 1936). Further discussion of the transmitter problem will be deferred to a later paper dealing with the action of eserine.

Time course of transmitter action. Whatever may be the nature of the agent producing the endplate potential, one can determine the probable time course of its action. It has been shown that most of the declining phase of the e.p.p. is a period of passive electrotonic decay. Since it follows an approximately exponential time course, one can analyze the e.p.p. on the basis of Hill's "local potential" theory (1936) and so obtain the probable time course of its underlying cause.

Making assumptions analogous to Hill's (1936), the endplate potential P , at any moment t , builds up at a rate (dP/dt) proportional to the intensity of the transmitter action cA at that moment (c being a proportionality constant), and simultaneously tends to decay exponentially with time constant k . P , k and dP/dt being known, cA can be calculated from

$$dP/dt = cA - P/k \quad (1)$$

In Fig. 19 the time courses of the e.p.p. and the depolarizing agent, as obtained by analysis according to equation 1, are illustrated. The duration of the depolarizing agent hardly exceeds the rising phase of the e.p.p., i.e.

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 about 2-3 msec. at 20°C. It will be shown in a later paper that eserine lengthens this period by more than 100 per cent.

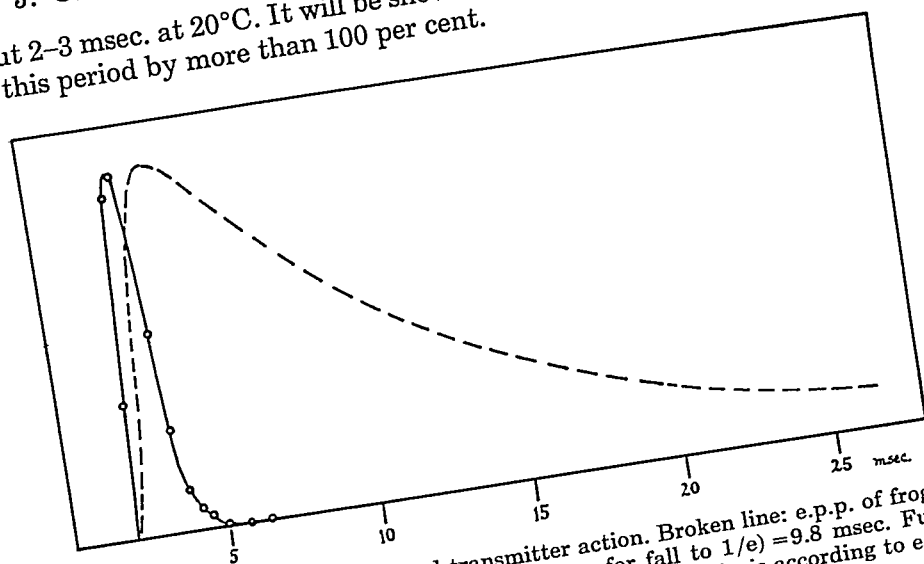


FIG. 19. End plate potential and transmitter action. Broken line: e.p.p. of frog's sartorius, at 17.5°C. Time constant τ of decay (time for fall to $1/e$) = 9.8 msec. Full line: Probable time course of "transmitter action," obtained by analysis according to equation (1). Ordinates in arbitrary units. Abscissae: Time after nerve stimulus.

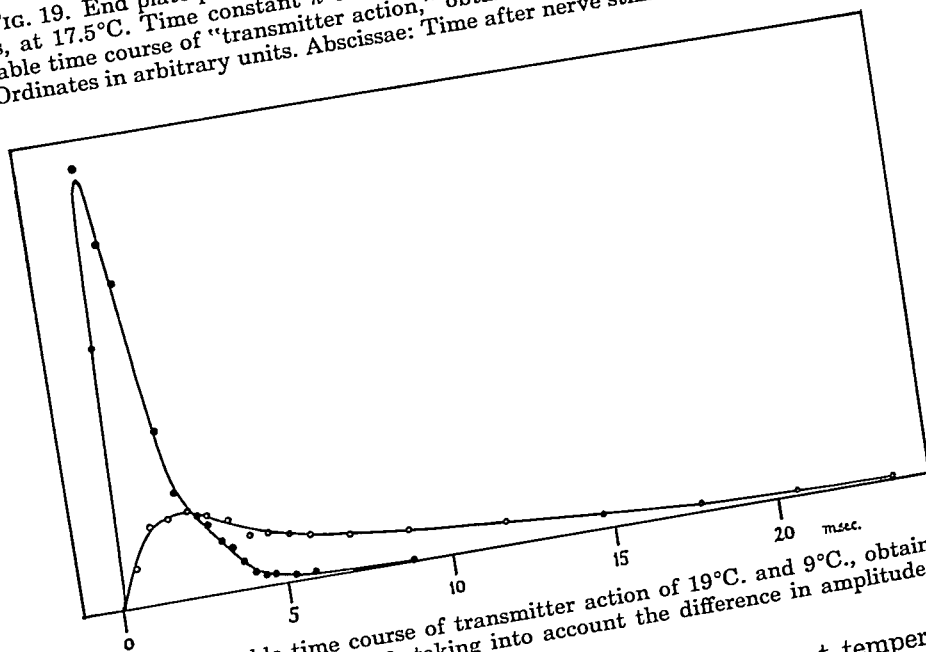


FIG. 20. Probable time course of transmitter action of 19°C. and 9°C., obtained by analysis of the e.p.p.'s of Fig. 8, taking into account the difference in amplitudes. Full circles: 19°C. Hollow circles: 9°C.

In Fig. 20 the e.p.p. has been analyzed at two different temperatures (cf. Fig. 8 above). The time course of the transmitter action, especially the rate of its decay, is greatly slowed at the lower temperature. Further, the

intensity of transmitter action is greatly diminished at the low temperature. The large temperature coefficient for the rising phase of the e.p.p., and so for the time course of transmitter action, might be taken as a further indication of its chemical nature. It should be remembered, however, that the duration and conduction velocity of the nerve or muscle spike have also a relatively high temperature coefficient (Q_{10} about 2.0, but occasionally reaching 2.6, cf. Table 3).

... does not consider the production of ... action currents ... ability. If acetylcholine, released, ... Id presumably be accompanied by a diminution in membrane impedance, and this would complicate the analysis. But even then the membrane must have returned to its resting state after a few milliseconds, as is indicated by the exponential decay of the e.p.p. and by the fact that a second e.p.p. can be added, at any moment of the descending phase, without alteration in time course (Section E).

Length of muscle fibre affected by transmitter action. Owing to electrotonic potential spread, a considerable portion of muscle is affected by the local changes produced by the transmitter. Though the direct action of the transmitter would be restricted presumably to an area not larger than the end-plate, the depolarization spreads over a length of about 2 mm. Moreover, in the frog's sartorius, most fibres are supplied by 2 or even 3 discrete motor nerve endings (Katz and Kuffler, 1941), so that in a muscle of 35 mm. length about 15 p.c. of the fibre length is affected by local events at the junctional regions. It is not surprising, therefore, that under certain conditions, when large and prolonged e.p.p.'s are set up (high-frequency nerve stimulation; eserine; see Feng, 1937; Eccles, Katz and Kuffler, 1940), the junctional change may be accompanied by a measurable increase in mechanical tension and heat production of the muscle (Feng, 1937; Cowan, 1940).

SUMMARY

In curarized frog's and cat's muscle a non-propagated negative potential change—the "end-plate potential"—is set up by a motor nerve volley at the region of the myoneural junctions.

By various tests, the end-plate potential (e.p.p.) is shown to be a local depolarization of the muscle fibres which acts like a local catelectrotonic potential produced by a subthreshold electric stimulus.

The e.p.p. spreads a short distance along the muscle fibres, with a decrement of about 50–75 per cent per mm., and a progressive slowing of its time course.

If recorded from the centre of an end-plate zone, in a state of just complete curarization, the e.p.p. reaches 5 to 8 per cent of the normal propagated muscle spike.

It waxes and wanes along an approximately double-exponential curve, the duration of the rising phase being 2.3 msec. in the frog's sartorius at

20°C., and 0.8 msec. in the cat's soleus, while the time constant of its descending phase is 11 msec. and 5 msec. respectively.

The temperature coefficient for the time factor of decay is low ($Q_{10}=1.3$), that for the duration of the rising phase is high ($Q_{10}=2.65$). The significance of this difference is discussed.

There is interaction between an e.p.p. and a propagated muscle-spike set up by direct stimulation. The e.p.p. sums with the "foot" of the muscle spike and slightly speeds up its propagation. During the "active phase" of the spike the e.p.p. is reduced or abolished.

In the frog a second nerve volley, at a brief interval after the first, sets up an e.p.p. which is up to 80–100 per cent larger than the first. This "super-normal" effect gradually disappears within about 0.1 sec. at 20°C. In the cat, the second e.p.p. is about 20 per cent smaller than the first and recovers its normal size only after several seconds.

If the e.p.p. exceeds a certain critical level, muscle spikes are initiated. Neuro-muscular facilitation occurs when the required level is reached by summation of successive e.p.p.'s.

We wish to thank the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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ISOLATION OF INTRINSIC AND MOTOR MECHANISM OF THE MONKEY'S SPINAL CORD*

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THE INTRINSIC MECHANISM of the spinal cord is compounded of nerve cells and nerve fibers: all the nerve fibers which both originate and terminate within the cord, and all the nerve cells giving rise to those fibers. The fibers in question for the most part compose the ground bundles or proprius system of the cord, running courses believed not to exceed seven segments up or down from their level of origin. But any of the long ascending fibers which send collaterals to the spinal grey matter must also be reckoned within the mechanism to the point, at least, where the last such collateral is given off. Correspondingly, the cells include small cells such as those in the substantia gelatinosa and the various sized association cells scattered throughout the grey which probably give rise only to intrinsic fibers, together with the larger cells which give rise to the long ascending systems but whose spinal collaterals enter into the system. Additionally, if any of the emerging ventral root fibers send recurrent (Golgi) collaterals back to the grey matter, then their cells of origin and fibers out to and including the collaterals form a part of the intrinsic mechanism. Excluded are the long descending systems, the entering fibers of the posterior roots, and the cells and fibers for the anterior roots when no collaterals are present.

To isolate the entirety of the intrinsic mechanism of the cord would require transecting the neural axis at the end of the medulla and severing all posterior and anterior spinal roots. This is scarcely practicable. To isolate a portion of the mechanism requires, however, only a double transection of the cord (one section high, one section low), and severance of the roots between. If only the sensory roots be cut, the motor roots being left intact, a preparation might be obtained in which many problems of function and structure in the intrinsic cord mechanism could be investigated provided only that the isolated region of spinal cord survived. That it can survive both the considerable trauma of the operation and being so isolated for a period of months has already been demonstrated for puppies (Tower, 1937), and some of the problems have been examined. The present report deals with a repetition of the experiment on 3 nearly grown monkeys (*Macaca mulatta*), killed, however, after 10 days or 2 weeks for chromatolysis studies on the isolated cord segments.

The surgical procedure consisted in exposing the lumbo-sacral spinal cord by removing the vertebral spinous processes and slitting the dura. The cord was then transected between the last thoracic and first lumbar segments and again below the sacral region, and

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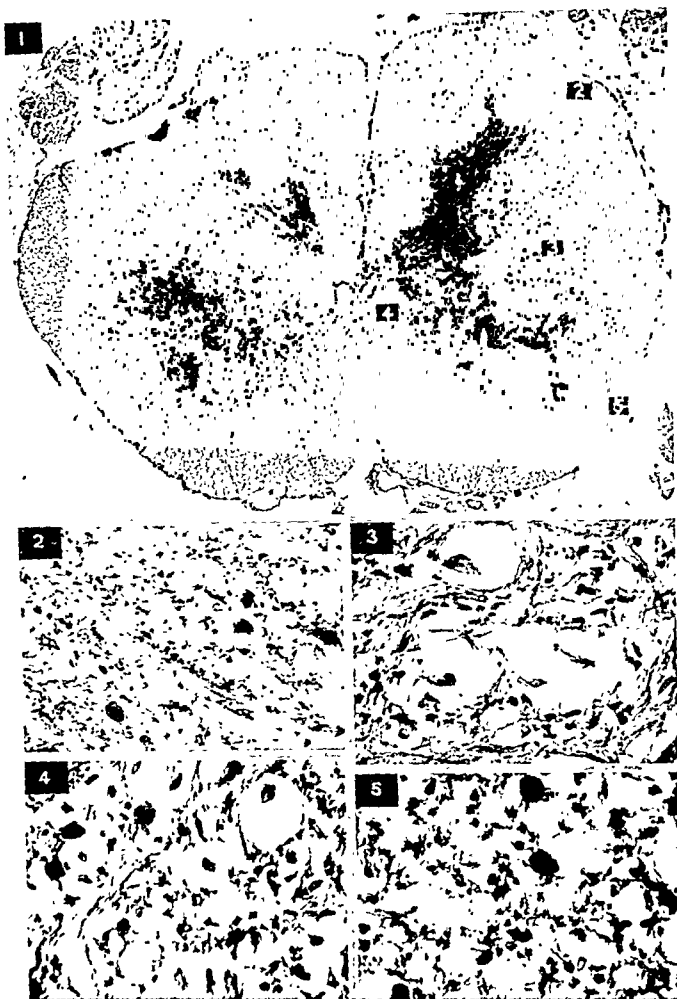


FIG. 1. Microphotograph of a section from the L_6 level of the completely isolated cord segment, stained with silver.

FIG. 2-5. Sample areas from Fig. 1 as indicated; oil immersion; $\times 870$. (2) from the lateral extension of Lissauer's zone; (3) from the lateral reticular formation; (4) from the deep end of the anterior column; (5) from the surface zone of the anterolateral column.

all intervening dorsal roots were cut intradurally on both sides, carefully sparing the anterior roots. The anterior spinal artery was preserved intact. Examined at autopsy, the isolated lumbo-sacral region of cord was found to be healthy and without blemish other than the intended sections in 2 monkeys, but liquefied in the third. In both surviving cords the upper transection fell between the T_{12} and L_1 segments and was complete, but the lower section was incomplete in one of the pair. This animal was killed on the 10th, and the other two on the 14th day of survival. In all 3 animals on the day following the cord operation the right sciatic nerve was exposed and cut near the greater trochanter without benefit of anaesthesia and without evidence of sensation on the animal's part. All the monkeys made uneventful postoperative recoveries, eating well and moving as much as their condition permitted. In spite of nursing care ulcerations developed in a number of sites, the largest over the right greater trochanter. Bladder and rectum had to be emptied by pressure. Terminally, under ether anaesthesia, the animals were killed and fixed by intravascular perfusion of 100 cc. of saline followed by 1 per cent acetic acid in 10 per cent formalin.

Examined in the interval of survival, all 3 monkeys were completely anaesthetic over the hind quarters, with a sensory level at the umbilicus or just below. In the animal which had an incomplete lower cord section, tail, perineal and genital reflexes were obtainable, but leg reflexes were absent. The other two animals yielded no skeletal reflexes whatever below the level of anaesthesia. The phrase "complete flaccid paralysis" describes the condition of the skeletal musculature innervated from the isolated segment, for no activity of any sort, neither tonic nor phasic, was observed in the musculature at any time after the operation, and no distinction could be made between the side with additional sciatic section and that on which the sciatic nerve remained intact. Moreover, prior to the final exposure of nerves and muscles there was no indication that the isolated segments of cord had survived the operation variously. Fasciculation was not observed in the affected skeletal musculature.

The two surviving isolated regions of cord were made the object of histological study in 10μ sections prepared both by Einarson's galloxyanin technique for Nissl substance and by Bodian's silver method for axis cylinders. From both cords the L_5 to L_7 segments were cut serially, transversely. From the completely isolated cord additional specimens from the lower part of T_{12} and the upper part of L_1 , that is just above and below the upper section, and a specimen from S_2 were cut transversely, and the whole L_4 segment horizontally. Similar transverse sections from normal monkey cords were available for comparison, together with an L_4 specimen obtained eight days after a lateral hemisection of the cord at the C_1 level.

Outstanding on first examination of the sections, L_1 excepted, are their good form both exteriorly and interiorly, and their approximately normal size. Later, presumably, shrinkage would have followed on the destruction of fibers, but the debris of that destruction is not yet entirely cleaned up, nor is sclerosis under way. Neuroglia are, however, unusually numerous and conspicuous in both white and grey matter. Figure 1 reproduces a cross section from the upper end of L_6 stained with silver. Although the trifling incompleteness of the caudal transection in one animal of the pair makes no noticeable difference at the levels examined, nevertheless both description

and illustrations are taken from the completely isolated specimen. The L_1 specimen, in contrast with the lower levels examined, is flattened and shrunk, with few cells surviving though many fine fibers, and with much evidence of widespread destruction.

Examining the silver preparations for nerve fibers:—The posterior roots are cut and degenerated proximally to the cut, but the anterior roots are intact. Occasional degenerated or degenerating fibers may be found in these roots but for the most part the fibers appear healthy. Within the cord the number of fibers surviving is so great that loss of fibers is at first glance scarcely appreciated until large myelinated fibers are sought. Healthy appearing fibers are distributed everywhere throughout the white matter, though far from evenly. Figure 6 represents diagrammatically the relative density of this distribution. Fibers are sparsest in the dorsolateral region of the posterior column where the medial division of the posterior root debouches into the cord. Indeed, this region is almost cleared of fibers. Next most depleted, but still possessing many small fibers, is the surface zone along the ventromedial curvature of the anterior column. The middle part of Lissauer's zone, the dorsal portions of the posterior columns, and the area occupied by the long descending motor systems of the lateral columns have also lost heavily in fibers. Adjacent to the grey matter everywhere except along part of the medial border of the posterior horn are fairly dense concentrations of fibers, occupying the dorsomedial third of the anterior column throughout its width, and extending to the surface of the cord in the ventrolateral region. The densest concentrations of fibers are found in three sites; lateral and medial to the tip of the posterior horn, although the small collection medial to the tip is not continuously present, and at the deep end of the posterior column. The large collection lateral to the tip of the posterior horn is the densest fiber field remaining in the cord.

The vast majority of the surviving fibers are small and seemingly unmyelinated, and for the most part they are clustered in the framework left after degeneration of the large myelinated fibers. The two dense areas at either side of the posterior horn are composed of the most minute fibers, most

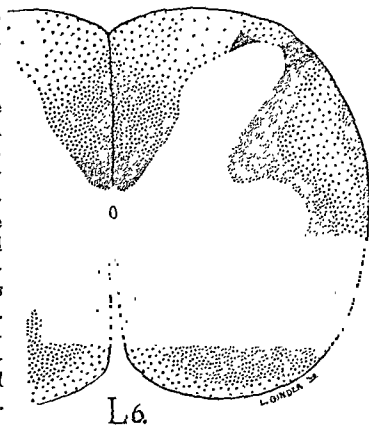


FIG. 6. Diagram representing the relative density of distribution of fibers surviving in the white matter of the completely isolated segment of spinal cord.

of them well under 1μ , and here the fibers are so closely and evenly packed that it seems unlikely these areas have suffered much, if any, fiber loss. Throughout the posterior column the fibers are very little larger, averaging around 1μ , but with a scattering of larger fibers, and the same is true of the dorsal parts of the lateral column. In contrast, beginning with the reticular formation in the angle between the dorsal and ventral horns, and bordering the grey matter around to the anterior commissure, the surviving fibers are of all sizes, ranging from a fineness almost too minute to be measured to fibers nearly as large as any to be found in the intact cord. Although the disappearance of many large fibers and consequent loose disposition of the survivors makes measurement difficult, diameters range from about 0.5 to 15μ for the poorly defined outside sheaths, and from 0.5 to 6μ for axis cylinders. The larger fibers are, of course, unmistakably myelinated, but myelin space may be recognized, though emptied in these silver preparations, on axis cylinders of 2μ diameter and perhaps less. Accompanying the cross section of the cord of Figure 1 are sample areas (Fig. 2-5) showing the characteristic fiber composition in four regions of the white matter.

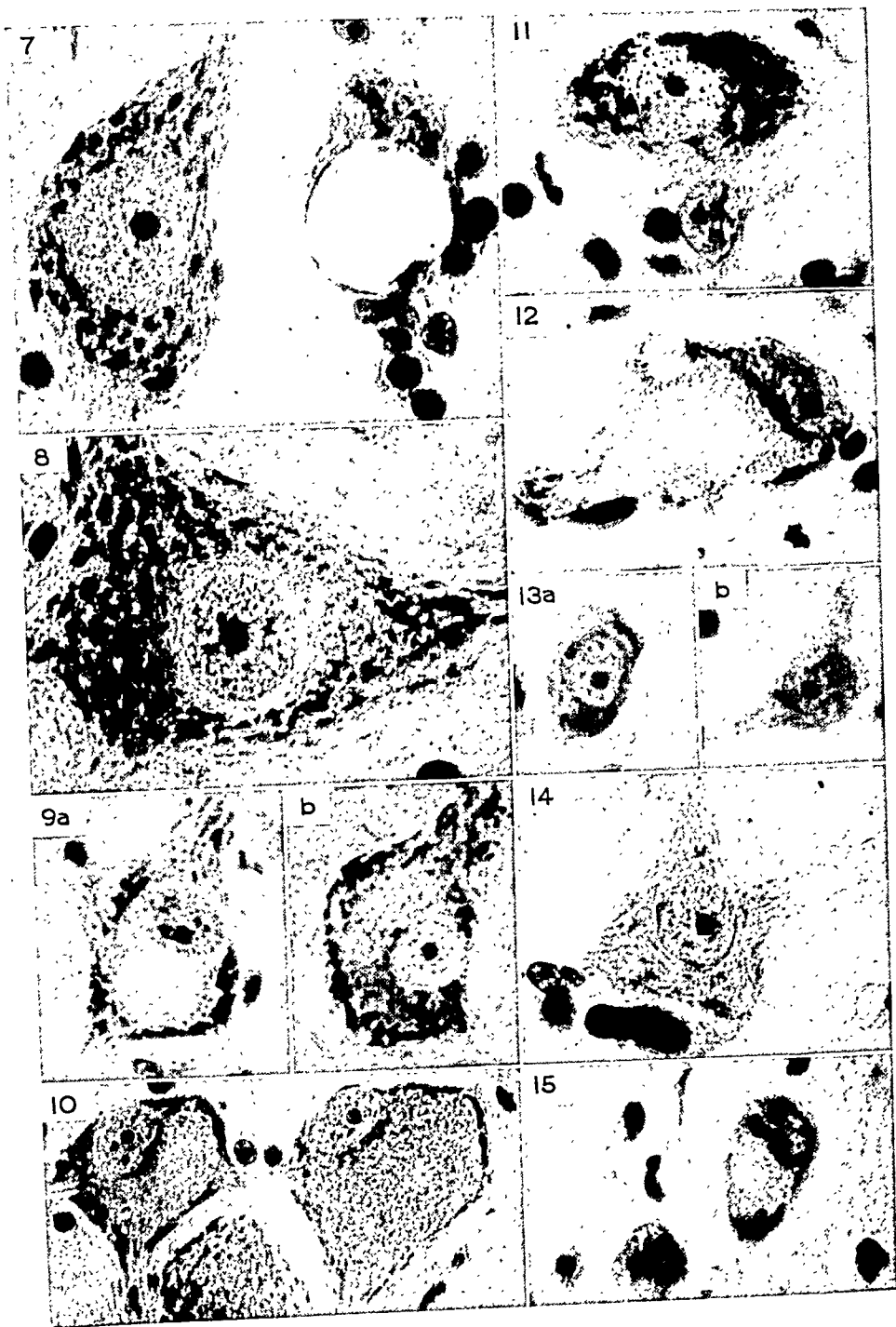
The presence of large fibers, fibers in excess of 10 or 12μ diameter, in the isolated length of spinal cord is noteworthy, especially so in view of Lloyd's (1941) demonstration that propriospinal fibers, as he calls them, with alpha conduction rates participate in aboral conduction within the spinal white matter. All levels examined except the much damaged L_1 level contain such fibers, although they are few at the S_2 level. Throughout the lumbar levels they constitute a numerous and impressive total aggregate. Two groups of these large fibers are outstanding; one in the reticular formation adjacent to the dorsolateral border of the anterior horn (Fig. 3) and extending around its dorsolateral tip; the other in the deep third of the anterior column (Fig. 4), beneath the anterior commissure. These latter fibers are traceable in some instances from the commissure, most clearly in the horizontal sections. Similar fibers are almost as numerous, cut in cross section, adjacent to the grey matter between the bundles of emerging ventral root fibers, but fewer along the lateral border of the ventral horn below the reticulated region. And in the horizontal sections the straight courses of individuals among these large fibers can often be followed for several millimeters, long enough to ensure that they are not emerging ventral root fibers. The course pursued by these large fibers is commonly lengthwise of the cord, but the direction, whether ascending or descending, is not evident. Different fibers may well course in opposite directions, and even one fiber in both directions if it is constituted as ascending and descending branches of a parent stem. Almost all these large fibers are found close to the grey matter, suggesting that whether or not any of them run through to the brain as a long ascending system, they all function importantly in the intrinsic cord mechanism. Throughout the superficial portions of the ventral and lateral white matter only an occasional large myelinated fiber is to be found, and the few present in the posterior columns lie deep, adjacent to the posterior commissure.

Similar large myelinated fibers, the largest, in fact, a shade larger, were described in the puppy cords isolated for months. There the fibers were distributed essentially as in the monkey except for more even scattering through the anterior and lateral columns adjacent to the grey matter, with an outstanding group only in the lateral reticular formation.

The fields of fibers beneath the surface of the lateral and ventrolateral white matter, which are fairly dense in the lumbar levels but sparse in the sacral, present a problem. They are composed almost exclusively of small fibers,—both unmyelinated fibers in clusters, and small myelinated fibers up to 5 or 7 μ over-all diameter (Fig. 5). Judging by the density of these areas in the isolated segments as compared with normal material, many of these fibers especially the myelinated individuals, must be components of the long ascending sensory systems which in the intact cord concentrate in these sites. Such fibers could very well survive up to the superior transection, for as will be shown later their cells of origin are largely intact. To what extent such fibers are to be considered components of the intrinsic system as originally defined, would be determined by whether or not they give off collaterals to the grey matter in their upward transit. Although this seems quite possible, the confusion of fibers is too great to permit of settling this point in reduced silver preparations.

Turning from the white to the grey matter but continuing the examination of fibers:—The neuropil appears, by comparison with normal material, definitely reduced in density. Outstanding even in Fig. 1 is the elimination of the heavy strands of root fibers or their collaterals which normally sweep into the dorsal horn from the dorsal root and column, but the intervening feltwork of fibers of the posterior horn is also unmistakably impoverished. Thinning is less noticeable in the anterior horn. Nevertheless the impressive aspect of the neuropil in both these sites is the number of fibers, both axonal and dendritic, surviving. And these fibers appear quite healthy and of a normal range of size except for certain pathological changes in the large dendrites to be described shortly.

The cells of the cord have for the most part also survived; and in larger proportion, on close scrutiny, than the fibers. But whereas the surviving fibers appear in all respects normal, the surviving cells are in many instances clearly pathological. Two types of disorder are in evidence. The more conspicuous is a condition of vacuolation barely visible in Fig. 1; the more subtle, chromatolysis in varying degree. These are distributed seemingly quite independently of one another throughout the grey matter. The vacuolated condition attacks only large cells, and in flagrant form, only anterior horn cells of the motor type, but attacks erratically so that two individuals lying side by side as in Fig. 7 may be: the one quite unaffected, the other almost overwhelmed by the process. No particular group of motor type cells is selected. The process consists in the formation of one or more large vacuoles, some as big or bigger than the nucleus, which may be located anywhere within the cell body or its larger dendrites, even bulging from the surface.



FIGS. 7-15

If the vacuoles are fairly small or few, the internal organization of the cell is not otherwise disturbed. Nissl bodies and neurofibrillae may be clear, and the nucleus normal in form and location. Well-formed Nissl bodies can be seen concentrated outside the vacuole in Fig. 7. Here and there, however, are frankly disintegrating cells, or the residue of completed cell destruction in accumulations of small round cells, probably phagocytes. Vacuolation was also the type of cell pathology present in the puppy cords after months of isolation, but its occurrence after other lesions, for example on both sides at the L₄ level after hemisection at C₁, and after root or even peripheral nerve sections, makes its interpretation obscure.

Chromatolysis is much more widespread throughout the isolated segments, attacking large and medium sized cells of both anterior and posterior horns in varying severity. Three types of distribution are present in the anterior horns. On the right side of the sections the proper cell groups in the L₅₋₇ segments exhibit the full-blown central chromatolysis with displacement of the nucleus to the periphery illustrated in Fig. 10, and to be expected 13 days after a sciatic section. These almost maximally chromatolysed cells may or may not be also vacuolated. Chromatolysis of this unilateral distribution is most evident in the last two lumbar segments. Headward of this in the L₄ segment, chromatolysis of equal severity has attacked numerous large cells scattered along the ventrolateral border of the grey matter—but on both sides of the cord. Occasional cells in a similar location in the L₅ and L₆ segments are similarly affected. Figure 9a shows one of these cells from the left side of the L₄ segment. This bilateral chromatolysis resembles in distribution that described by Cooper and Sherrington (1940) consequent on hemi- or transection of the cervical or thoracic cord,

Fig. 7-15. Untouched microphotographs of cells stained for Nissl substance. On the left, anterior horn cells; on the right, posterior horn cells. Oil immersion.

Fig. 7. Normal and vacuolated cells lying side by side in the left anterolateral group of L₇. Satellite cells have accumulated around the vacuolated nerve cell but its Nissl bodies are still clear. $\times 870$.

Fig. 8. Large cell from the left posterolateral group of L₇, showing a minimal degree of perinuclear palor. $\times 870$.

Fig. 9. Chromatolysis in presumed spinal border cells at L₄; a) from the left side of the completely isolated cord segment; b) from the right side eight days after a left hemisection at C₁. $\times 530$.

Fig. 10. Cells from the right posterolateral group of L₇, showing grave chromatolysis as the result of the right sciatic section. $\times 530$.

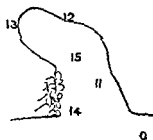
Fig. 11. Healthy cells in the medial-basal portion of the left posterior horn; L₇. The large cell is probably one of those composing Stilling's nucleus. $\times 870$.

Fig. 12. Severely chromatolysed cell from the medial margin of the left posterior horn; L₇. $\times 870$.

Fig. 13. Healthy pair of cells from the apex of the left posterior horn; L₇. $\times 870$.

Fig. 14. Healthy cell of the type characteristic of the basal group of the posterior horn; left side; L₇. $\times 870$.

Fig. 15. Chromatolysed cell lying just ventral to the substantia gelatinosa in the nucleus of the posterior horn of the left side. L₇. $\times 870$.



and interpreted by them as evidence that these anterior horn cells, so-called spinal border cells, give rise to a decussating, ascending system which they believe to be Gowers' tract. Figure 9b reproduces a cell from the ventrolateral part of the anterior horn of the L₄ segment on the side opposite a hemisection at C₁ of 8 days' standing which should be one of these spinal border cells.

With few exceptions, severely chromatolysed anterior horn cells fall into one or the other of these two groupings, but lesser degrees of chromatolysis are widely distributed throughout the anterior horns. Figure 8 illustrates the most prevalent condition: a perinuclear zone of pallor without displacement of the nucleus or other disorder, and of doubtful pathological significance. This condition is distributed among the anterior horn cells quite haphazardly, selecting neither the very largest nor any particular group, and altogether about half the cells are in some degree affected. Again, both cells in this condition and those free of it may or may not be also vacuolated.

In contrast with the anterior horn cells as a group, the posterior horn cells are for the most part healthy in appearance; the small cells of the substantia gelatinosa entirely so. Of the large and medium sized cells whose long ascending axons were presumably cut by the upper transection, the majority are not visibly chromatolysed, but a not inconsiderable minority show a typical, more or less grave reaction (Fig. 12 and 15), and occasional cells appear on the verge of destruction. Reviewing the various groups of sensory cells: the pericornual cells, both marginal and apical, are less often attacked than the large cells occasionally present in the substantia gelatinosa and composing a central nucleus in the dorsal horn just below, whereas sparse but conspicuous cells of different morphology, probably to be considered Stilling's group, are almost never chromatolysed unless some of the cells so gravely affected as to be unrecognizable as to type are of this group. Generally speaking, when chromatolysis attacks individuals of these groups, it tends to be severe. In contrast is the group of cells most numerous affected. This occupies the full width of the basal part of the posterior horn, extending laterally into the reticular formation, and perhaps also anteriorly to include cells in the dorsomedial group of the anterior horn which are of similar type. About a third of these cells of variable size but characteristic morphology, presumably association cells, are chromatolysed, especially the medial lying members, but the reaction is typically moderate and unaccompanied by displacement of the nucleus. Figures 11 to 15 illustrate seemingly normal individuals from these groups and two examples of chromatolysis. Vacuolation of the flagrant type attacking the anterior horn cells, does not occur in the posterior horn, nor in the dorsomedial group of the anterior horn. Figure 12 shows the modest vacuolation occasionally present in these cells.

SUMMARY

This brief study demonstrates the feasibility of isolating regions of mature spinal cord from all ingoing nerve impulses and utilizing them for exam-

ination of various questions in neuro-anatomy and neuro-physiology. It has given a picture of the intrinsic and motor mechanism of the spinal cord cleared of posterior root and descending fibers, showing the magnitude, variety and arrangement of the intrinsic system in the monkey. Finally, it has confirmed the conclusion reached after very much longer study of isolated segments in dogs, that the mammalian cord mechanism operates only under the stimulus of arriving nerve impulses. Deprived of such excitation the cord produces no activity which reaches effectiveness in the skeletal musculature.

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FIBRILLATION IN SKELETAL MUSCLE IN RELATION TO DENERVATION AND TO INACTIVATION WITHOUT DENERVATION*

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SKELETAL MUSCLE, when left in possession of its innervation but rendered inactive for a period of months by cutting off the innervating region of spinal cord from all ingoing nerve impulses, atrophies and becomes contracted (Tower, 1937). Examined in the intact living animal, or grossly at autopsy, there is nothing to distinguish this reaction from the reaction of muscle to denervation. Examined histologically, however, the reaction differs from denervation atrophy in the failure of the subsarcolemmal nuclei either to alter their form or to proliferate. In the final stage of fibrous metaplasia they simply disappear. The motor end-plates likewise remain virtually intact until this stage. Denervation atrophy and inactivation atrophy, or atrophy of disuse, thus appear to be related but not identical processes. In the study referred to, however, the living muscle was never exposed and directly examined for the occurrence or absence of fibrillation.

Now, with increasing appreciation of the far-reaching consequences of denervation, suggested especially by the finding that with series innervations such as those of the autonomic nervous system the effects of denervation may be evident trans-synaptically (Cannon, 1939), the possibility looms that isolation of the spinal cord may have constituted a functional denervation of the skeletal muscle such that fibrillation followed. In that case the attendant atrophy could scarcely be considered to have been the result of simple inactivation. The preparation of three adolescent monkeys with isolated lumbo-sacral spinal cords as described in the preceding communication, each with an additional right sciatic section, offered an opportunity to determine whether muscle rendered inactive but left in physical possession of its nerve supply is in fact as inactive as it appears to be; or whether, like denervated muscle, it fibrillates. The prior experience of others (Matthes and Ruch, 1931; Sherrington, 1932; Cooper and Sherrington, 1933; Fulton and McCouch, 1937) with paraplegic monkeys, which has shown the liability of this species to traumatic degeneration of the sciatic nerves, makes the monkey not the animal of choice for such a test, but as the results turned out, they are significant.

The observations for fibrillation were made terminally on two of the three monkeys in which the inactivation of the hindquarters was complete. Except for a difference in sensory level amounting to one segment, the condition of these two animals during survival was in every respect similar even

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to an ulcer developing over the right greater trochanter and not over the left. In both, the skeletal musculature below the level of lesion was atonic, completely paralysed, and uncontractured. To what extent the muscles could be considered atrophied at the time of death is uncertain for both animals had lost weight, the legs losing more in bulk than the arms. Certainly, however, both animals and both legs of each animal shared in the loss fairly equally, and the loss was not yet great.

The first indication that the condition of the two animals was not identical came to light only in the terminal examination. On the fourteenth day after the cord operation, the thirteenth after the sciatic section, muscles were exposed on the front and back of the thigh and lower leg and the interosseous muscles of the foot, and examined with a hand-lens for fibrillation. No anaesthesia was required for this procedure. In the first animal examined, the muscles of both sides were found to be fibrillating actively from thigh to foot. The sciatic nerves of the two sides were then exposed, the right in the thigh, the left near the greater trochanter. Both nerves were swollen and yellowish, and electrically inexcitable (60 cycles; 8 V). It was concluded therefore, that both nerves were degenerated, the left probably in consequence of degeneration of the isolated cord segment. This conclusion was verified at autopsy when the segment was found to be completely necrotic.

In the second monkey, similarly examined, the muscles of the right leg were similarly fibrillating from thigh to foot with the exception of those on the anterior surface of the thigh, but the muscles of the left leg were entirely at rest. The fibrillating muscles of the right side were strikingly deeper red in color than the corresponding quiet muscles on the left, and deeper red on the whole than those of either arm. The right sciatic nerve, exposed in the thigh, was swollen, yellowish and electrically inexcitable whereas the left nerve, exposed near the greater trochanter, was of normal appearance, and when stimulated (60 cycles; 1 V), caused the muscles of the calf and foot to contract quickly and strongly. It was concluded that the isolated cord segment in this animal must be in good condition, a conclusion which was again confirmed by autopsy and subsequent microscopic examination of the cord. A cross section from this isolated region of cord stained with a silver stain, may be examined in the preceding paper, together with specimens of the anterior horn cells of the two sides stained for Nissl substance.

Cutting the spinal cord off, literally, from all ingoing nerve impulses constitutes, therefore, neither a denervation of the isolated cord tissue such that this becomes spontaneously active, nor a functional, trans-synaptic denervation of the dependent and still anatomically innervated skeletal muscle such that this enters into fibrillation. The apparent quiescence in both cord and muscle seems to be real. If this be true, then the conclusion previously reached is valid—that the atrophy described under similar conditions previously is atrophy of inactivity, or so-called atrophy of disuse. By this should be understood, however, only that inactivity is the setting

for the atrophy, the mechanism of which remains at the present time still to be analysed.

The problems presented by the atrophies of skeletal muscle become more and more challenging. The apparent inactivity of denervated muscle, which had much to do with developing the general concept of atrophy of disuse, has been shown to be unreal. Denervated muscle is ceaselessly active in the minute contractions of fibers or of parts of fibers called fibrillation, and this activity continues certainly for a year (Tower, 1939a) and possibly for many years after the denervation, so long, probably, as the muscle survives as a contractile tissue. Reviewing recently (Tower, 1939b) the evidence bearing on the mechanism of denervation atrophy, weight of evidence seemed to support Langley's view, that denervation atrophy, far from being a consequence of disuse, is a consequence of exhaustion of the muscle by the continuing fibrillation. Since then, however, Solandt and Magladery (1940; in part confirmed by Ravin, 1940) have succeeded in preventing the fibrillation of denervation by the use of drugs, and have shown that the atrophy continues. Is this, then, atrophy of disuse? And have the two conditions a common basis even though atrophy of inactivity appears the simpler histologically (Tower, 1937; Tower, 1940)? Such a common basis might be sought in disturbance of an optimal tension as was long ago suggested. Or more in line with current thought, it might be sought in the cessation of neuromuscular transmission which follows equally on section and degeneration of the nerves and in circumstances of total inactivity. The neurotrophic influence thus brought to an end might conceivably be either a simple conditioning effect, or the result of substance liberated; the latter possibly, but by no means necessarily identical with the humoral transmitter, acetylcholine. Carried further for a longer survival period, though preferably not on the monkey, the experiment here described, with denervation of one extremity and inactivation of the other, may offer an opportunity to compare the morphology, physiology and chemistry of the two atrophic processes in skeletal muscle, with results of consequence for the understanding of atrophy.

SUMMARY

To investigate the possibility that surgical isolation of the spinal cord from all ingoing nerve impulses might constitute a functional denervation of the dependent skeletal muscle such that this enters into fibrillation, the lumbo-sacral region of cord was so isolated in two monkeys and in addition, one sciatic nerve was cut in each. Two weeks thereafter leg muscles were exposed on the two sides and examined for fibrillation. Muscle still in possession of its innervation was found to be at rest, not fibrillating; but muscle denervated either by sciatic section, or, in one animal, in consequence of complete degeneration of the isolated cord segment, was found to be fibrillating and of a deeper red color than the muscle at rest. It is concluded,

therefore, that atrophy developing under conditions of inactivation without denervation may properly be considered inactivation atrophy, or atrophy of disuse.

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INITIATION OF MUSCLE IMPULSES AT NEURO-MUSCULAR JUNCTION

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A PREVIOUS PAPER (Eccles, Katz and Kuffler, 1941) has dealt with the end-plate potential (henceforth called e.p.p.) set up by a nerve volley in completely curarized muscle. It has there been shown that a spike potential, due to a propagated muscle impulse, arises when the e.p.p. is caused to surpass a certain critical value, either by allowing some recovery from the curarization, or by summation of the e.p.p.'s set up by two or more nerve volleys. It is thus clear that in partly curarized muscle the e.p.p. forms an essential link between the nerve impulse and the initiation of the muscle impulse. In this paper the relationship of e.p.p. to initiation of muscle impulses will be examined for normal muscles. This investigation would be best made on an isolated single nerve-muscle fibre preparation, but, as this does not at present seem feasible, we have used the innervated strip preparation of the cat's soleus, and have also performed some parallel experiments on the frog's sartorius.

The experimental procedures have already been described (Eccles and O'Connor, 1939; Eccles, Katz and Kuffler, 1941). Special care has been taken to maintain constancy of temperature, which has often been within 0.2°C . for hours. However it will be seen that some curare experiments proved difficult in this respect, as curarization is associated with a considerable fall in the cat's metabolism, and hence with the fall of a previously stabilized temperature. The necessary detailed analysis has been obtained by photographing at high speed (up to 27.5 m. per sec.) and at high amplification, and the curves have been measured with a lens to 0.05 mm. and plotted at 20 times magnification.

A. EXPERIMENTAL RESULTS

In locating the endplate zone on an innervated muscle strip of the cat's soleus, the "recording" electrode is moved up or down the strip until the latency of the spike peak is at a minimum (usually about 1.5 msec.). Then slight sideways movements to the position for maximum spike potential ensure that the electrode is placed centrally on the endplate zone of the strip. In preparations with a sharply localized endplate focus the action potential so recorded rises steeply to a summit which passes over to a less steep decline, thus resembling in its general course the nerve spike potential. However considerable differences are observed in the initial part of these muscle potentials: in some preparations they begin abruptly with a very brief initial "foot" (Fig. 1a)—the "simple-spike" type; in others there is an initial step clearly separated by an inflexion from the steep rising phase of the spike (Fig. 1b)—the "double-step" type; finally Fig. 1c shows an intermediate

type where an initial slow rise is not clearly separated from the steep rise of the spike.

There are two probable explanations of the initial step shown in Fig. 1b and c: it may be the spike potential of a small group of muscle fibres whose endplates are nearer to the recording electrode than the main focus of endplates; alternatively the initial step may be e.p.p., which in these experiments would attain a considerable size before the spike is set up. Three tests have been employed in distinguishing between these alternative explanations, i.e. between e.p.p. and spike, and also in investigating the difference between the double-step and the simple-spike potentials.

(i) The effect of graded doses of curare. The spike is delayed and eventually abolished, while the e.p.p. is progressively diminished but not delayed.

(ii) The changes in the action potential produced by small shifts of the recording electrode from the endplate focus along the strip. The spike potential shows a time shift due to conduction with but little alteration in size, while the latent period of the e.p.p. is but little altered and it rapidly decrements.

(iii) Determining by subtraction (cf. Eccles and O'Connor, 1939) the potential added by a second nerve volley at various intervals after the first volley. An early second volley sets up an e.p.p. uncomplicated by muscle spike, and at longer volley intervals the spike arises from the initial e.p.p., all transitions to the normal action potential being observed.

1. *Graded curarization.* It has already been shown that in curarized muscle both the latent period and time course of the e.p.p. are not significantly altered when it is greatly depressed by further curarization (Eccles, Katz & Kuffler, 1941). This suggests that in normal muscle a similar latent period and time course would also obtain for that part (if any) of the e.p.p. which precedes the advent of the spike, and should also be observed at all intermediate stages of curarization—subparalytic, partly paralytic and paralytic.

A rough preliminary comparison may be made by superimposing on the normal action potential the e.p.p. set up in complete curarization, the respective amplifications being chosen to facilitate matching of the initial step with the e.p.p. This comparison (Fig. 2) reveals a striking similarity with

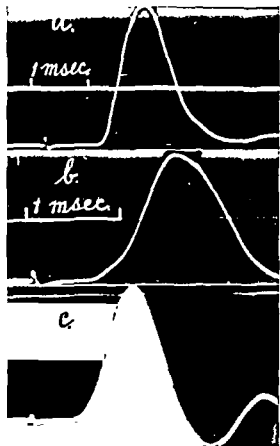


FIG. 1. Cat's soleus: action potentials which a nerve volley sets up at the endplate zone of an innervated strip in three different experiments. Time scale of c same as a.

both latent period and time course. A more detailed comparison has been made by plotting on the same time-deflection co-ordinates the action potentials set up by a single nerve volley at all grades of curarization (Fig. 3). The normal action potential at 13.2 times less amplification is also plotted so as to show its initial phase of similarity with the pure e.p.p. (cf. Fig. 2b). In order to establish a relationship between the e.p.p. and the initial step, we must show that the time courses of the curves are similar, and we must also determine if there is a time shift of the curves due to any alteration

which curare might produce in the latent period. This is best done as follows.

On one curve an arbitrary point is chosen, e.g. B in Fig. 3, and the time BA is measured for doubling this potential, BC for halving it, etc. On each other curve points $\beta\alpha$ are chosen which by trial have the same doubling time BA (0.083 msec.). It will be seen that on the curves where this is feasible the β points lie at about the same time as B. Furthermore, if from these β points the γ points be found for the respective half potentials, these γ points again lie close to the time of C on the original curve—similarly for δ points, etc. In this way the initial parts of the lower series of curves in Fig. 3 are shown to have similar curvatures at any given time; they can be accurately matched by suitable ordinate scaling. Similarly, standardizing by the doubling time $\gamma\beta$ (0.055 msec.), the highest curve of this first series matches with those curves too high for the determination of a doubling time of 0.083 msec. In this way by using, as it were, relays in the matching, the initial parts of the whole family of curves can be

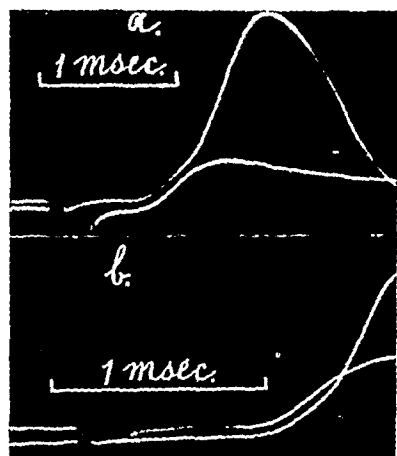


FIG. 2. Superposition of the normal action potentials of Fig. 1b and c (Fig. 1c taken with a faster time base) on the e.p.p.'s set up after complete curarization. These latter potentials are recorded at 9.3 and 13.2 times the amplification in order to give approximate matching with the initial step of the normal potentials. The stimulus artefacts are synchronized and the records are shown slightly separated to facilitate comparison.

shown to be accurately matchable simply by choosing suitable ordinate scaling. In Fig. 3 the extreme range of the variations in latency is only about $10 \mu\text{sec.}$ and there is no systematic change with curarization.

This matching provides strong presumptive evidence that the initial phase of all these curves is an e.p.p., which is diminished in size by curare, but not altered in latency—exactly as occurs in completely curarized muscle. The normal action potential at low amplification (curve ABC) supports these conclusions, for just beyond A it deviates sharply from the e.p.p. curve (shown by the broken line), a deviation which presumably is due to the muscle spike. Thus in this experiment it would seem that the normal action potential is initially an e.p.p., and, when this potential attains a

value about 13 per cent of the peak of the muscle action potential (henceforth called the peak-potential), the muscle spike is set up giving a suggestion of a double step. The amplification is too high to show the origin of the spike in the other records of Fig. 3.

The spike origin is well seen in Fig. 4, which gives a similar family of curves for another double-step experiment in which matching is also possible

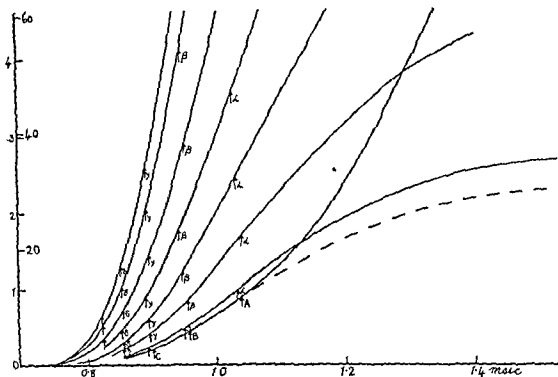


FIG. 3. Rising phases of action potentials set up at endplate zone by a nerve volley during progressive curarization, the highest curve being the normal response. All are plotted on the same time scale and the potential scale (percentages of normal peak-potential) shown to the left obtains for all except the lowest record, which is a normal action potential at 13.2 times less amplification (cf. Fig. 2b). The two next lowest curves are pure e.p.p.'s, and the broken line gives the same time course for the initial endplate potential of the normal curve, the deviation due to the spike origin being well shown. See text for the significance of the letters in relation to the detailed matching of the curves.

by choosing suitable multiplication factors. In addition to showing a constant latency of the e.p.p., Fig. 4 indicates that *pari passu* with progressive curarization there is a lengthening latent period of the spike. This lengthening is best measured by the shock-peak times. Thus in Fig. 4 the normal shock-peak time of 1.45 msec. is lengthened to 1.57 msec. with a subparalytic dose of curare, to 1.80 msec. with a considerable paralysis, and finally to 2.0 msec. with the last muscle fibre to resist paralysis. In every experiment the maximum lengthening of this shock-peak time has exceeded 0.25 msec. (cf. Fig. 5).

One other "double-step" experiment has given a family of curves similar to those of Fig. 3 and 4, and in another the test described for Fig. 3 revealed a slight temporal shift with progressive curarization—in all about 0.06 msec. Probably this shift was due to slight cooling rather than to an exceptional action of curare in delaying the time course of the e.p.p. A cooling of 0.6°C.

would account for this shift, and, as the cat's temperature fell by 1.5°C . while the box temperature did not vary more than 0.2°C ., such a cooling of the muscle possibly occurred.

Thus in these four experiments there is good evidence that the e.p.p. has formed the initial step, the relatively late origin of the spike giving the main potential wave—a conclusion fully confirmed in sections 2 and 3. When the initial step of the action potential is due to an early spike response of a

few muscle fibres—the alternative explanation suggested above—progressive curarization delays and eventually abolishes both the initial step and the main spike, leaving only the small e.p.p. electrotonically transmitted from the endplate zone (cf. Eccles, Katz and Kuffler, 1941). The deviation from the continuous matching series shown in Fig. 3 and 4 is so striking that such action potentials are easily distinguishable from the true double-step potentials with their initial e.p.p. step.

Figure 5 illustrates the effect of progressive curarization in a "simple-spike" experiment (observation 1). Subparalytic curarization delays the muscle spike, which now seems to take off from an initial step (observations 2 and 3). With deeper curarization (observations 4 to 9) the spike is further delayed and the initial step more obvious. Finally observations 10 and 11 show that the time course of the pure e.p.p. corresponds closely to that of the initial step, and this is seen more clearly in the corresponding plotted curves of Fig. 6. Match-

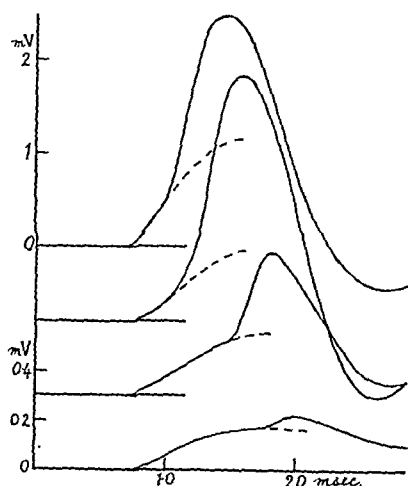


FIG. 4. Potentials set up by a nerve volley at endplate zone, plotted to show progressive delays of spikes with increasing curarization (top record normal). The potential scales for the two upper records and the two lower records are shown separately. The broken lines indicate the continuation of the initial e.p.p. step.

ing tests such as those applied to Fig. 3 reveal that the initial rising phases of observations 3 to 11 (Fig. 5) match with those of observations 1 and 2, but have a latency about 0.05 msec. longer. This lengthening was due to temperature change, for it occurred while the temperature fell about 0.4°C . and the curarization was constant. Hence these matching tests indicate that the initial step in the partly curarized potentials has the same latency and time course as the e.p.p., and even the normal action potential seems to have a slight initial phase of e.p.p.

The smallness of this initial step would thus provide the only difference between this simple-spike experiment and those double-step experiments illustrated in Fig. 1, 2, 3 and 4. Whereas in Fig. 5 and 6 the spike deviation from the e.p.p. normally seems to occur when this has only attained about 1 per cent of the peak-potential, it occurred at the much higher values of 6.5,

12, 13 and 15 per cent in the 4 double-step experiments. However another experiment was transitional, for a clear double-step was observed though the spike arose when the initial e.p.p. had only attained 3 per cent of the peak-potential. Four other experiments have conformed to the type of Fig. 5 and 6, matching tests indicating a spike origin when the initial e.p.p. had attained no more than 1-2 per cent of the peak-potential.

In all these experiments the matching tests at high amplification allow us to measure the steepness of the initial phase of e.p.p. relative to its steepness in completely curarized muscle. It is then possible to calculate the maximum to which the e.p.p. would normally rise if the spike did not supervene. Table I, column 2 shows that with one exception this maximum lies between 30 and 42 per cent of the peak-potential and that there is no significant difference between experiments with large and small initial phases of e.p.p.

Finally there were two exceptional "simple-spike" experiments (cf. Fig. 1a) in which the matching tests failed. Thus, in Fig. 7, observations 1, 2 and 3 cannot be matched with the initial e.p.p. step which appeared in the deeper curarization of observations 4 to 6, where matching was possible as in Fig. 3. The origin of the spike is clearly seen in observation 5, and the matching test reveals its origin in observation 4. If observations 1, 2 and 3 begin with an e.p.p., then the initial dose of curare must have lengthened its latency and also altered its course, effects which curare does not have when the e.p.p. is certainly recognizable in this and other experiments. It is therefore probable that, in these first 3 observations of Fig. 7, the spike arose before the e.p.p.; a small initial e.p.p. step (about 1 per cent peak-potential) first appeared when this spike origin was delayed about 0.1 msec. by curare. With further curarization this experiment resembled that of Fig. 5. However it is not possible to exclude an exceptional action of curare in lengthening the latency of the e.p.p.

Conclusions. Detailed study of the effects of progressive curarization on the rising phase of the normal action potential provides good evidence that two distinct actions are involved.

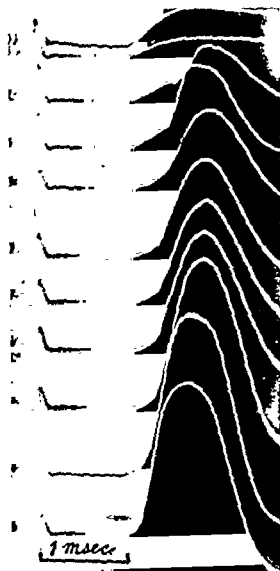


FIG. 5. Observation 1 is normal action potential at endplate zone (simple-spike type) and series shows effect of progressive curarization; 11 is the pure e.p.p. at 2.7 times amplification of observations 1 to 7, and 8, 9 and 10 are 1.7 times.

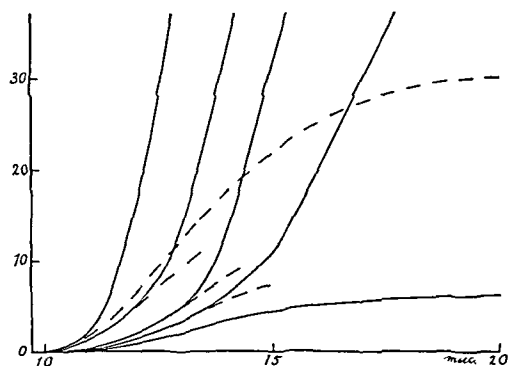


FIG. 6. Plottings of observations 1, 2, 3, 7 and 11 of Fig. 5 on the same potential-time coordinates. Ordinate scale: percentages of normal peak-potential. The continuations of the initial e.p.p.'s are shown by the broken lines in order to indicate spike origins.

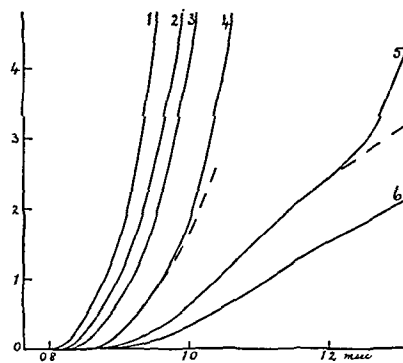


FIG. 7. Plotting as in Fig. 6 of another experiment in progressive curarization. The first three curves cannot be matched by any multiplication of the e.p.p. Temperature maintained constant throughout series: cat 39.0°C., box 38.0°C.

(a) Progressive diminution of the e.p.p. without altering its latency or time course, *i.e.* this part of the potential is matchable by suitable ordinate scaling.

(b) Progressive lengthening of the spike latency by at least 0.25 msec., and its eventual diminution and extinction.

On this basis our experiments fall into three groups. (a) In five the e.p.p. forms the initial step which attains from 3 to 15 per cent of the peak-potential before the spike supervenes. (b) Five other experiments resembled group *a* in having an initial phase of e.p.p., but the spike supervened before this had attained 2 per cent of the peak-potential, and no initial step was observed. (c) Finally in two experiments the spike appeared to arise before the e.p.p.

With groups *a* and *b* there is no significant difference in the rate of rise of the initial e.p.p.; hence they are only arbitrarily distinguishable according as the initial e.p.p. is or is not large enough to give a step which is differentiated by an inflection from the later spike origin. Moreover a little curare converts group *b* into group *a*, and even group *c* into *b* and then into *a*, the distinctions merely arising according to the earliness of the spike origin relative to the e.p.p.

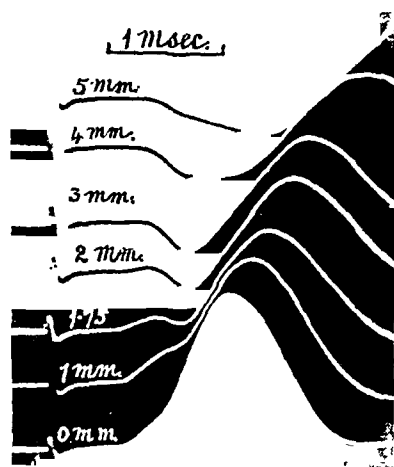


FIG. 8. Action potentials set up by single nerve volley and recorded at the endplate zone (as located by initial test), and at the indicated distances in millimetres along the strip from this zone. The shift has a differential effect on e.p.p. and spike.

2. *Shifts of the recording electrode along the muscle strip.* In Fig. 8 the recording electrode has been moved along the innervated strip to positions successively more proximal than the endplate focus. Correspondingly the spike potential is progressively more delayed. Figure 9 shows a direct proportionality of the shift (either proximal or distal) to lengthening of the spike latency and indicates a velocity of 3.2 m. per sec. for propagation of the muscle impulses from the endplate zone in both directions. On the other

hand the initial e.p.p. step shows no alteration in latency with increasing shifts, but it diminishes in size and eventually seems to disappear beyond 3 mm. from the endplate zone. This was confirmed by records at five times greater amplification. However, when the recording electrode is 1.75 mm. or more from the endplate zone, the muscle impulses arising there given an initial phase of positivity (cf. Eccles and O'Connor, 1939, p. 49). With the 1.75, 2 and 3 mm. positions this initial phase of reverse spike recording cuts into the e.p.p. giving a sharp positive dip between it and the spike. Beyond 3 mm. the e.p.p. itself shows the reverse recording already described (Eccles, Katz and Kuffler, 1941), adding its positivity to that of the spike.

There is a similar gamut of changes when the recording electrode is moved from the endplate zone distally along the muscle strip. For example in Fig. 9 the arrows proximal and distal to the endplate zone mark the points beyond which the positive dip would cross the zero potential line. The action potentials of Fig. 8 are not significantly changed by altering the position or area of contact of the relatively indifferent electrode.

Two other double-step experiments have given series of potentials differing from Fig. 8 only in having about half the spatial scaling. Thus in the double-step experiments the e.p.p. does not reverse sign until the recording electrode is moved from the endplate zone to about twice the distance required for reversal of spike potential. This difference appears to conflict with the conclusions that the e.p.p. is a depolarization of the muscle membrane and that it spreads electrotonically along the membrane similarly to the foot preceding the muscle spike (Eccles, Katz and Kuffler, 1941). However during the spike the great diminution in membrane impedance (Cole and Curtis, 1939) presumably would alter the circuits responsible for the reverse recording, and so may diminish the distance at which reversal of the potential is observed.

Whatever the explanation, the difference in the critical distances for reversal is useful in distinguishing between the spike and the e.p.p. Thus in

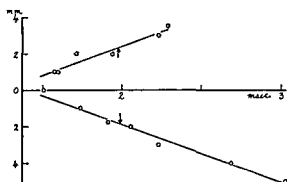


FIG. 9. Distances proximal and distal from endplate zone plotted as ordinates against latent periods of spike crests as abscissae. The oblique lines give a velocity of 3.2 metres a second, and suggest that the centre of the endplate zone is about 0.2 mm. distal to the point determined by initial test. The proximal points are plotted below the endplate line and are derived from Fig. 8.

Fig. 5 graded curarization indicated that the spike supervened when the e.p.p. had attained only about 1 per cent of the peak-potential; correspondingly a shift of only 0.75 mm. from the endplate zone sufficed to give a small initial positive deflection due to inverse recording of the spike, which was so early relative to the e.p.p. that no initial phase of e.p.p. negativity could be

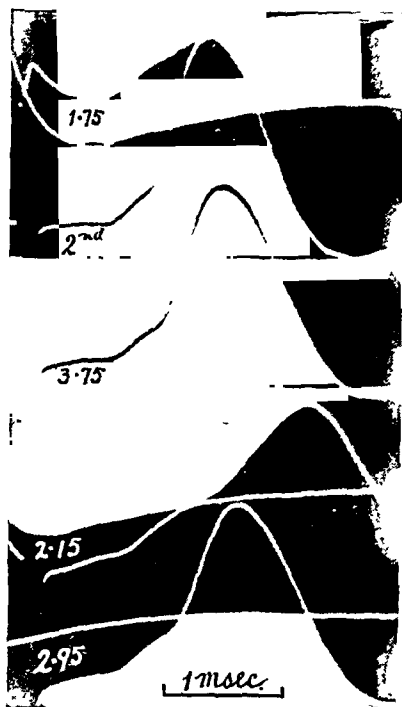


FIG. 10. Action potentials added by second nerve volley at the indicated intervals in milliseconds after the first nerve volley. The base line formed by the response to first volley alone is shown just above or below the double response. The second stimuli are fired at a constant position on the sweep. The spike origins from the initial e.p.p. steps are well shown; no spike with the 1.75 msec. interval.

zone of a frog's sartorius and attribute the first step to the e.p.p. However they have not based this identification on any tests such as the three described in this paper, and, moreover, their first step is so large (up to 80 per cent of the peak-potential) that it is very unlikely to be an e.p.p. preceding the spike origin. Presumably, as with the similar potentials of our frog's sartorii, their double-step potentials arise on account of two adjacent foci of endplates.

detected. With graded curarization, however, the spike origin is delayed (cf. Fig. 5), and correspondingly 0.75 mm. away a negative e.p.p. is then seen preceding the spike, its height being about 40 per cent of that at the endplate zone.

Successive small shifts of the recording electrode along the innervated strip, therefore, distinguish between the e.p.p. and the spike potential and support the conclusion derived from the curarization experiments—that in some experiments a large initial e.p.p. step precedes the spike, while in others this initial potential is small or possibly absent. This method of investigation is also important in identifying the spurious initial step which is produced by the spike potential of a small focus of endplates nearer to the recording electrode than the main focus; the spurious step shows time shifts due to alterations in conduction time.

Apparent double-step potentials were frequently observed in frog's sartorius, but small shifts of the recording electrode showed invariably that both steps were due to propagating spikes arising from two discrete points on the muscle. In many other cases simple spikes were recorded at the endplate zone (see Katz and Kuffler, 1941, Fig. 1, 2, 3, 4, 8, 9, 10. Schaefer and Haass (1939) claim that double-step action potentials are always present at the endplate

3. *Rising phase of action potential set up by an early second nerve volley.* It has already been shown (Eccles & O'Connor, 1939) that an early second nerve volley sets up a pure e.p.p., and that with longer volley intervals a spike also arises, all transitions being observed to the action potential set up by a single nerve volley. Such a series of observations form the basis of the present test for distinguishing between the rising phases of the e.p.p. and the spike in the normal action potential. The requisite degree of accuracy is obtained by employing faster recording and higher amplification as shown in Fig. 10 for a "double-step" experiment.

The potential added by the second nerve volley at each volley interval is obtained by subtraction (cf. Eccles and O'Connor, 1939, p. 65) and plotted on the same potential-time coordinates (Fig. 11), the second stimulus

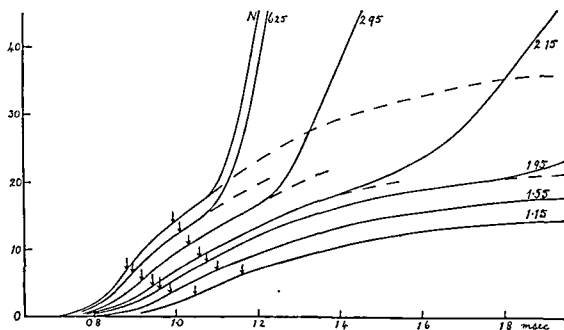


FIG. 11. Potentials (as percentages of normal peak-potential) added by second nerve volley to first response at the volley intervals indicated in milliseconds (N is normal response). With 1.15 and 1.55 msec. intervals, the added response is an e.p.p., but with longer intervals spikes are also set up later (cf. this same series in Fig. 10). The initial e.p.p. steps are continued as the broken lines. Between the arrows each curve doubles in height in 0.115 msec. (cf. matching test of Fig. 3). Other parts of the e.p.p. curves conform with this matching.

artefact giving zero time. The presence of all transitions and the general similarity of time courses strongly suggest that the e.p.p. which alone is set up at short volley intervals is also alone responsible for the initial step of the normal action potential in Fig. 10 and 11. Now the matching tests already used in Fig. 3 show that the curves of Fig. 11 can be matched provided that allowance is made for the longer latencies at the shorter volley intervals (cf. Eccles & O'Connor, 1939, Fig. 22). Thus the initial phase of all the potentials is a pure e.p.p. and the spike can be seen to arise abruptly from it, the spike latency diminishing to the normal value with lengthening of the volley interval.

The maximum height of the e.p.p. set up by an early second volley can be directly measured and expressed as a percentage of the normal peak-potential; hence the matching of the potentials makes it possible to calculate the height to which the initial e.p.p. step of the normal action potential would attain if extrapolated beyond the spike origin (cf. the upper broken line of Fig. 11). The value of 36 per cent of the peak-potential thus calculated for Fig. 11 is in satisfactory agreement with the value of 42 per cent obtained by the graded curarization test later in this experiment. Similarly, in two other double-step experiments, the responses to any second volley match closely with the initial step of the normal action potential, and are in good agreement with the effects of graded curarization.

The size of the e.p.p. may be plotted against the corresponding stimulus interval, or, more significantly, against the response interval (Fig. 12),

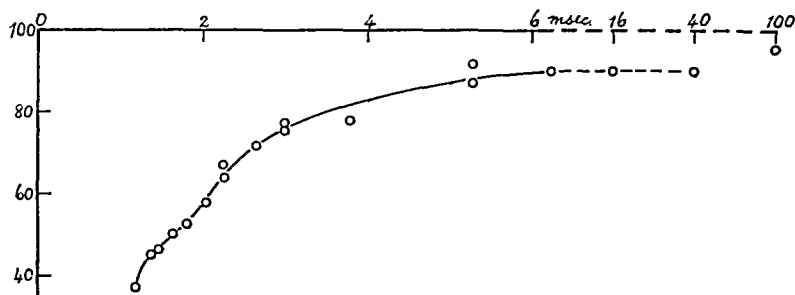


FIG. 12. Experiment partly shown in Fig. 10 and 11. Ordinates, size of e.p.p. set up by 2nd nerve volley as percentage of normal e.p.p.: abscissae, response intervals, *i.e.* intervals between corresponding points (see arrows, Fig. 11) of e.p.p.'s set up by 1st and 2nd volleys.

allowance being made for the lengthened latencies (as indicated by arrows in Fig. 11). With short volley intervals the e.p.p. is diminished to less than half its normal size and recovery is largely complete when this interval is lengthened to 5 msec.

In Fig. 13 the potentials set up by a second nerve volley in a simple-spike experiment have been plotted as in Fig. 11. Again at the shorter volley intervals there is accurate matching of the pure e.p.p.'s, but with longer intervals the early spike origin restricts the matched region to a small initial part of the curve. Thus with the normal action potential the spike arises when the e.p.p. is not more than 3 per cent of the peak-potential, and the rate of rise of this initial e.p.p. indicates that, if extrapolated, its maximum would attain 27 per cent of the peak-potential. There is satisfactory agreement with the test by graded curarization, which indicated that there was an initial e.p.p. phase of about 2 per cent and an "extrapolated maximum" of 35 per cent.

Similarly in three other simple-spike experiments matching of the added e.p.p.'s showed that the spike was set up after an initial e.p.p. phase of

about 2 per cent and there was an "extrapolated maximum" of 26 to 30 per cent of the peak-potential.

The matching of the curves of Fig. 13 allows the size of the e.p.p. to be plotted against the response interval just as in Fig. 12. The curve so obtained (Fig. 14) closely resembles that of Fig. 12, and other experiments of this type have also given similar curves. This similarity confirms the general conclusion derived from the graded curare tests—that the experiments giving normally action potentials with the simple-spike and double-step type are only arbitrarily distinguishable according as the initial e.p.p. is or is not large enough to form a discrete step.

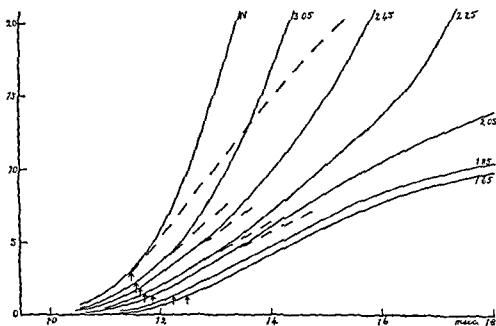
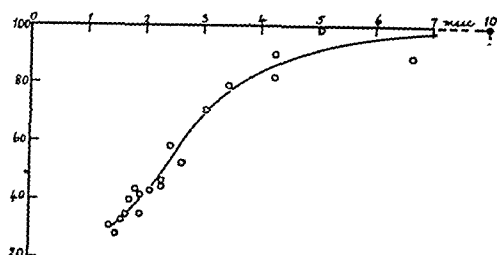


FIG. 13. As in Fig. 11, but in a simple-spike experiment. The arrows mark corresponding points on the e.p.p. curves as determined by matching. The broken lines show the continuation of the initial e.p.p. phases along courses matching the pure e.p.p.'s at the two shortest volley intervals.

The curves of Fig. 12 and 14 have some theoretical significance. In another paper (Eccles and Kuffler, 1941) it will be shown that an early second nerve volley sets up a diminished e.p.p. on account of the refractoriness of the muscle—an effect similar to that observed for the e.p.p. in completely curarized muscle (Eccles, Katz and Kuffler, 1941). The responses of completely curarized muscle show that, in the absence of muscle refractoriness, a second nerve volley more than 1.5 msec. after the first has a practically constant excitatory action in setting up an e.p.p. (Eccles, Katz and Kuffler, 1941, Fig. 17a). Hence the curves of Fig. 12 and 14 express recovery from the muscle's refractoriness, as measured by its ability to respond by an e.p.p. to the approximately constant excitatory action of the second nerve volley.

Figures 12 and 14 show that, 1.5 msec. after the response to the previous volley, a second nerve volley sets up an e.p.p. only 30–45 per cent of the normal value. There is a suggestion of an initial phase of slower recovery up to about 1.8 msec., and then recovery of the muscle is practically linear

to 3 msec., where the response is 65–75 per cent normal. At 6 msec. recovery may be almost complete (Fig. 14), or a small deficiency may remain for some



time—up to 100 msec. in Fig. 12. This prolonged deficiency recalls the diminished e.p.p. set up by a second volley in curarized cat's muscle (Eccles, Katz and Kuffler, 1941), but the recovery time is there very much longer—3 to 10 sec.

FIG. 14. As in Fig. 12, but for the experiment partly shown in Fig. 13.

B. DISCUSSION

From the three tests described above it is clear that all transitions

exist between experiments in which the normal action potential shows a large initial e.p.p. step (12 to 18 per cent of the peak-potential) and those where the spike is initiated so soon that the e.p.p. has risen to not more than 1–2 per cent of the peak-potential—or even possibly has not risen at all. Further, these latter simple-spike types are converted into the double-step type either with subparalytic curarization (Figs. 5, 6 and 7) or with the action potentials set up by the earliest second nerve volleys to initiate spikes (Fig. 13).

As shown in columns 2, 3 and 4 of Table 1, the extrapolated heights of the normal e.p.p. and the fractions to which it is diminished with just subparalytic ($\frac{2}{3}$ to $\frac{3}{4}$) and half-paralytic curarization ($\frac{2}{5}$ to $\frac{1}{2}$) are consistent and show no significant relationship to the size of the initial phase of e.p.p. (column 5), *i.e.* to the simple-spike or double-step type of action potential.

When the initial e.p.p. is large (12–15 per cent of the peak-potential—early in experiment 18/4/40 it was 18 per cent), progressive curarization is associated with a spike origin at lower e.p.p.'s (columns 6 and 7), and complete paralysis (column 8) is not produced until the summit of the e.p.p. is less than 40 per cent of the initial e.p.p. step (*cf.* Fig. 3 and 4). It is probable that the actual threshold potential remains approximately constant at any one endplate, because curare has little or no effect either on the electrical excitability of muscle or on the propagation of muscle impulses. However, with progressive curarization, the following factor would cause the spike to be initiated at a lower observed value for the e.p.p.

A given dose of curare will diminish to varying degrees the e.p.p.'s at different endplates. This would in part arise on account of varying concentrations of curare, as determined for example by the relation of the endplates to the blood vessels. Also in some experiments pressure of the recording electrode might seriously have interfered with the circulation to part of the endplate zone. Presumably muscle spikes would be set up first at those endplates least affected by curare, and so would arise when the aggregate e.p.p. is less than the threshold potential obtaining normally in the absence of this unequal depression by curare.

This factor is probably adequate to account for the spike origin at dimin-

Table 1. Columns 2, 5, 6, 7 and 8 are expressed as percentages of peak-potential

Experiment	"Extra- polated maxi- mum" for normal e.p.p. 2	Rate of rise of e.p.p. (as fraction of nor- mal rate)		Height of e.p.p. at spike origin			Maximum height of e.p.p.— just complete curariza- tion 8
		Just sub- paralytic curariza- tion 3	Half paralytic curariza- tion 4	Normally 5	Just sub- paralytic curariza- tion 6	Half paralytic curariza- tion 7	
1							
9/2/40	30			3			4.5
13/2/40	37	$\frac{1}{2}$	$\frac{2}{3}$	13	6	5	5
16/2/40	?			20	2.5	2.5	3
23/2/40	41	$\frac{1}{2}$	$\frac{1}{2}$	6.5	4.5	2.5	3.5
27/2/40	35			1			3
7/3/40	?			20		1.5	6
13/3/40	35	$\frac{2}{3}$	$\frac{1}{2}$ to $\frac{1}{3}$	2	1.5	2.5	2.5
15/3/40	30	$\frac{1}{2}$	$\frac{1}{2}$ to $\frac{1}{3}$	12	7	3	2.6
18/3/40	30	$\frac{2}{3}$	—	1 to 2	4		5.5
18/4/40	42	$\frac{2}{3}$	—	15	9		5.5
13/5/40	50		$\frac{1}{2}$	1.5		2.5	

ishing e.p.p.'s in columns 5, 6 and 7 of Table 1. Thus in these three experiments the e.p.p. is probably the sole mechanism initiating the muscle impulses both with normal and with subparalytically curarized endplates. On that basis the safety factor obtaining for normal neuro-muscular transmission would be approximately given by the ratio of the values of column 2 to those of column 5. Thus the safety factor for each of these three experiments is about 3. Alternatively the safety factor for the average neuro-muscular junction may be derived from the diminution in e.p.p. necessary for half paralysis. Under such conditions there would be an approximately symmetrical distribution of the endplates with respect to the just paralytic intensity of curarization, and the aggregate e.p.p. would be diminished to about the degree which is critical for interrupting neuro-muscular transmission at the average neuro-muscular junction; hence the reciprocal of the diminution shown in column 4 would give the average safety margin. The values of 2.5 and 3.5 so derived for the safety margin in experiments 13/2/40 and 15/3/40 are in good agreement with value of about 3 calculated by the previous method.

On the other hand in Table 1, columns 5, 6 and 7 show that, in experiments with normally a very small initial phase of e.p.p., progressive curariza-

tion is associated, on the whole, with a spike origin at an increasing e.p.p. (cf. Fig. 5 and 6). Finally column 8 shows that with a just paralytic degree of curarization the e.p.p. is practically as large as with the double-step experiments. This progressive increase would be accentuated by the above factor. Thus these observations suggest that, at any one endplate, the e.p.p. is normally very small when the muscle impulse is initiated, and this critical size becomes increasingly larger as the curarization deepens—a condition which is improbable if the e.p.p. is the sole factor initiating the muscle impulse. Again with these experiments the above calculations of the safety factor show a wide discrepancy—a value of 15 or more being given by the first method above, and 3 to 5 by the second. Finally we have one and possibly two experiments (16/2/40 and 7/3/40) in which the muscle impulse may have been initiated even before the e.p.p.

Thus, with this group of experiments, where the initial phase of e.p.p. is normally small or even absent, there is probably some additional agent initiating muscle impulses. In other respects we have seen that these experiments appear to be similar to the above three experiments (experiment 23/2/40 is probably intermediate), *e.g.*, there is a similar extrapolated maximum for the normal e.p.p. (column 2), and a similar height for the e.p.p. at just complete paralysis (column 8); thus they differ only in that they appear to have an additional exciting agent superimposed on the e.p.p. mechanism. The increase shown in columns 5 to 6 to 7 to 8 for this group indicates that with progressive curarization the e.p.p. takes an increasing share in the initiation of the muscle impulses; hence the additional exciting agent is probably depressed *pari passu* with the e.p.p. The simplest suggestion is that the action currents of the motor nerve impulses exert this additional exciting effect, and the anatomical relationship of the nerve fibre to the motor endplate and the adjacent muscle fibre might account for the great variability from one experiment to another. Against such a suggestion is the depressant action of curare, which is not observed with direct electrical excitation of muscle fibres, though it must be remembered that the endplate zone is a specialized region of the muscle.

Thus it may be concluded that in a few experiments a nerve impulse normally initiates the muscle impulse by setting up the intermediary e.p.p., and there is no evidence for any other excitatory action on the muscle fibre. More often there appears to be an excitatory action additional to the e.p.p., and in a few experiments this additional mechanism may even be solely responsible for normal neuro-muscular transmission, the e.p.p. mechanism being revealed only when the additional mechanism is depressed by curare or by the refractory period following a previous muscle response. We have no evidence relating to the nature of such an additional mechanism, and no sign of its subliminal action can be seen. Possibly this exciting mechanism is analogous to the detonator action described for synaptic transmission (Lorente de Nó, 1935, 1939; Eccles, 1937, 1939), and it may be caused by the direct excitatory action of the nerve action currents on the muscle fibre at the region of the neuro-muscular junction.

SUMMARY

A detailed study has been made of the rising phase of the muscle action potential which a nerve volley sets up at a focus of endplates. In some preparations these potentials are of the "simple-spike" type, in others an initial step precedes the spike (the "double-step" type).

Three procedures have been used in identifying that component of the rising phase due to "endplate potential" (e.p.p.): progressive curarization; small shifts of recording leads away from the focus; responses added by a second volley at various intervals.

It is thus shown that the initial step of the double-step potentials is due to e.p.p., the spike only arising when this has attained a considerable size—up to 16 per cent of the peak-potential. With the simple-spike potentials there is usually also an initial e.p.p. phase, but it is small—usually 1–2 per cent of the peak-potential. All transitions are observed.

There is no significant difference between the e.p.p.'s of the two types: in both the initial steepness of e.p.p. rise indicates an extrapolated maximum of about 30–40 per cent peak potential; in both curare and the muscle's refractory period depress the e.p.p. similarly. The difference solely concerns the earliness of the spike origin relative to the e.p.p.

Large initial e.p.p.'s (12–18 per cent peak-potential) are probably the sole mechanism initiating muscle impulses both normally and during sub-paralytic curarization or refractoriness of the muscle. The normal safety margin is about 3.

In the other experiments the excitatory action of the e.p.p. seems normally to be forestalled by an additional excitatory mechanism, which is depressed by curare or refractoriness, the e.p.p. then taking an increasing share in excitation. The nerve action currents may be directly responsible for this additional excitatory effect.

We wish to express our thanks to Dr. Bernhard Katz for his valuable suggestions and to the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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THE HUMAN ELECTRO-CORTICOGRAM

A REPORT OF SPONTANEOUS ELECTRICAL POTENTIALS OBTAINED FROM THE EXPOSED HUMAN BRAIN*†‡

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I. INTRODUCTION

ELECTRO-ENCEPHALOGRAPHY, or the recording through the intact skull of spontaneous electrical potentials generated by the brain, has assumed during the past two or three years increasing importance as a clinical aid in the discovery and localization of intracranial disease (1, 2, 3, 4).

It seems self-evident, however, that the ultimate value of the clinical encephalogram must be based upon a secure knowledge of the electro-physiology of the underlying brain, which can only come from precise observations taken directly from the exposed brain. An appreciable amount of work has been done upon the electrical activity of the exposed cortex of animals; and these accumulated observations are quite valuable for comparative study (5, 6). However, even for the purpose of studying normal human physiology, observations made from the brains of experimental animals are no substitute for observations made directly from the human brain. Moreover, the difficulty of reproducing in laboratory animals pathological processes comparable to those found in man practically precludes animals as a source of knowledge about the electrical activity of the brain in the presence of disease,—especially the neoplastic, degenerative and convulsive diseases. For such information one must study directly the exposed brains of sick patients.

Electrical potentials recorded directly from the human brain have been reported in only a few instances (7, 3, 8). This paper describes electrical activity obtained by us directly from human brains exposed at the operative table, in patients suffering from a variety of intracranial diseases.

II. METHOD OF STUDY

Electro-corticograms were taken during eighteen operations performed upon seventeen patients suffering with a variety of organic intracranial lesions. The cortex in the cases

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‡ Case reports and figures are given in full in the reprints which may be obtained from the authors.

studied was exposed by the usual operative technique for craniotomy. Wherever possible, local anesthesia alone was used although it was necessary to do a certain number of the cases under avertin anesthesia. In a few cases it was necessary to supplement the avertin with very mild temporary inhalation anesthesia of ether. In one patient, a small child, primary ether anesthesia was used throughout. Details regarding anesthesia have been included in the individual case reports. The exposed cortex was first stimulated electrically, principal motor points marked by tags placed directly on the brain, and operative field photographed. Electro-cortical potentials of the exposed brain were then studied. In the first three cases, unipolar recording was employed; but in all of the subsequent cases, recording electrodes (Fig. 1). These bipolar electrodes were made of light, coiled, silver wire mounted on a form of mechanical stage which permitted fast, accurate adjustments in all directions. The electrode assembly was sterilized in the autoclave.

The electrodes were never allowed to pierce the pia or the cortex because the injury potentials so evoked unnecessarily would complicate the observations (10). Initial records

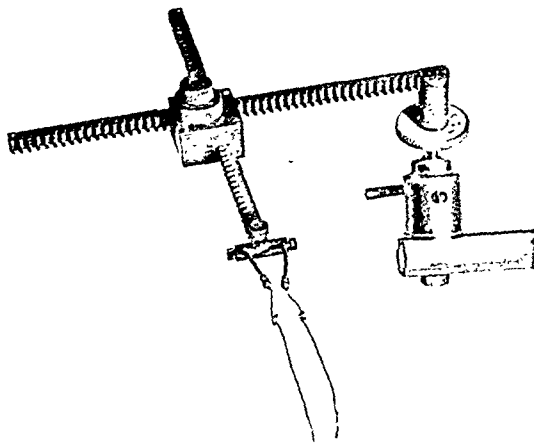


FIG. 1. The bipolar electrodes here shown were developed early in the study, and were used for recording on all except the very earliest of the cases. These electrodes were made of light, coiled, silver wire mounted on a form of mechanical stage which permitted fast, accurate adjustments in all directions.

Great care was always taken to prevent the tips of the electrodes from piercing the pia mater, since it has been shown that this is a frequent source of false potentials.

were usually taken prior to any surgical manipulation of the cortex. Subsequent records from cut surfaces of brain tissue were also made in cases which afforded this opportunity.

Shielding in the form of a single sheet of copper screen was placed under the patient during the preparations for the operation. The copper sheet and the amplifying system were both grounded. It was necessary to disconnect the patient from the electro-cautery machine prior to recording and also curtail the use of extension cords and other electrical appliances in the operating room. Records of the cortical potentials were made by means of suitable amplifiers and dynamic ink-writing recorders, mounted in a small and mobile

cabinet which was easily handled in the operating room. This equipment constructed by one of us (W.E.R., Jr.) has already been technically described (9). An accurate record was always kept of the pulse and respiratory rates during the recording and all records subsequently eliminated where synchrony existed between apparent rhythmic electro-cortical activity and these rates.

The only serious artefacts which arose were caused by gross movement of the electrodes incident to pulsations of the brain. These artefacts could in most instances be eliminated by applying the electrodes so that the points move perpendicularly with the pulsations rather than making a sliding movement over the cortical surface. The introduction of coils in the electrode arms was found to increase their flexibility and greatly reduce these artefacts. (Fig 1).

III. OBSERVATIONS

(A) *Classification and regional distribution of spontaneous rhythmical potentials.* In the categorical consideration of spontaneous brain potentials we have used the following classifications. Potentials having a frequency less than eight cycles per second are called "slow waves," and on the basis of empirical findings are considered to be abnormal. Those potentials ranging from eight to twelve cycles per second are considered to be alpha waves. The average frequency of the alpha rhythm is ten cycles per second. Rhythmic variation higher in frequency than twelve cycles per second are classed as beta potentials. Both alpha and beta potentials are considered to constitute normal rhythms.

In the cases we have studied, no absolutely constant localization of spontaneous rhythms could be demonstrated. It would appear, however, that the *alpha* rhythm is confined chiefly to the parietal, temporal, and occipital lobes. In only one case have we observed potentials of alpha frequency in the frontal lobes. The central sulcus appears to be the anterior limit of the alpha rhythm. Our finding of the alpha rhythm in the temporal and parietal lobes confirms the work of Berger (12), and Jasper and Andrews (13), and is at variance with the findings that the alpha rhythm is exclusively occipital in origin. The alpha rhythms ranged in frequency from eight to twelve cycles per second with an average frequency of ten cycles per second.

Beta activity is the dominant rhythm in the frontal lobes and though this rhythm may appear in the temporal or parietal lobes, when this occurs the amplitude is usually greatly diminished as compared to that in the frontal region. So that while there is a wide individual variation in the amplitude of beta potentials, this relative difference in amplitude between the frontal lobes and other portions of the brain is usually maintained. This rhythm is also more pronounced in the anterior than in the posterior portions of the temporal and parietal lobes. The observed beta rhythms showed a range of from thirteen to twenty-two cycles per second with an average frequency of eighteen cycles per second.

Illustrative examples of the *alpha* rhythm are to be found in Case I, ‡ Fig. 3, graph 2; Case VI, Fig. 9D; Case VII, Fig. 10E; and Case XV Fig. 21, graph 16. Examples of the *beta* rhythm are given in Case I, Fig. 3, graph 1; Case IV, Fig. 7A; Case V, Fig. 8B and C; Case VII, Fig. 10E; Case VIII, Fig. 11A; Case IX, Fig. 12B; Case XI, Fig. 14A and B; Case XII, Fig. 15A;

Case XIII, Fig. 17B; and Case XIV, Fig. 19, graph 9. Examples of *beta* activity arising posterior to the central sulcus are found in Case VIII, Fig. 11A; and Case XII, Fig. 15A. The single instance in which *alpha* activity was obtained anterior to the central sulcus is not among the illustrative cases used in this paper.

The optic nerves anterior to the chiasm yielded rhythmic potentials of *alpha* frequency in one case of our series in which it was possible to place a recording electrode directly upon one of the optic nerves (Case II, Fig. 4B). Electric potentials recorded from the cerebellum, in one case (Case III, Fig. 5) were difficult to interpret, and do not permit any generalizations regarding them to be made.

Slow waves were obtained from damaged areas of the cortex regardless of the anatomical location. They appear to be merely an expression of physiological impairment, and depend for their production solely upon injury to nerve tissue. They are affected more by the degree of injury than by its nature or by the cytoarchitecture wherein the injury occurs.

(B) *Tumor tissue is electrically inactive regardless of type of tumor.* The electrical activity of tumor tissue was investigated directly at the operating table in eight patients. The tumors studied included three glioblastomas, one astrocytoma, one meningioma, one craniopharyngioma, and two metastatic carcinomas.

In not a single instance was spontaneous electrical activity obtained directly from neoplastic tissue.

Illustrative examples of these recordings are to be found in Case IV (glioblastoma) Fig. 6 and 7B; Case V (astrocytoma) Fig. 8A; Case VI (meningioma) Fig. 9B; Case VII (metastatic carcinoma) Fig. 10D.

(C) *Abnormal electrical potentials, usually associated with brain tumors, arise not from tumor itself, but from damaged brain tissue immediately adjacent.* This is clearly demonstrated in Case IV, Fig. 6 and Fig. 7C; Case V, Fig. 8B and C; Case VI, Fig. 9D; Case VII, Fig. 10E. The infiltrating tumors (gliomas) characteristically produce severe and widespread changes, both anatomic and physiologic, in the surrounding brain, thus giving rise to electro-cortical dysfunction which is correspondingly great (Case IV, Fig. 7C; Case V, Fig. 8B and C). The maximum disturbances are observed in those regions of the cortex which are being most actively invaded by the tumor (Case V, Fig. 8B). At greater distances from the tumor the abnormal potentials are gradually replaced by the normal rhythmic activity of that part of the brain (Case V, Fig. 8C).

The encapsulated tumors (meningiomas), on the other hand, usually produce very little physiologic disturbance in the adjacent brain, with the result that these tissues usually reveal amazingly little electro-cortical dysfunction, even though the tumor itself may be quite large (Case VI, Fig. 9D).

The frequencies of the spontaneous rhythms arising from tissue in the vicinity of the diffuse infiltrating tumors are shifted downwards as compared with the frequency of those rhythms characteristic of normal tissue in the

same brain. On the other hand, the encapsulated benign type of neoplasm, by reason of the limited abnormal potential changes induced, shows no lowering in the frequency of the brain rhythms arising in the tissue adjacent to the tumor. These frequency shifts are easily seen if the alpha frequency is measured carefully in tissue near the tumor and at a distance therefrom.

It is often possible, therefore, to distinguish between the infiltrating and the encapsulated tumors not only by the magnitude of the electro-cortical dysfunction and its topographical extent, but also by the degree to which the frequency of spontaneous cortical rhythms have been slowed down.

As cortical tissue becomes increasingly damaged, however, a point is finally reached, as might be expected, where all electrical activity ceases. This is well shown in Case VII, Fig. 10B.

(D) *Changes in electric potentials in association with internal hydrocephalus.* In two patients with increased intracranial pressure secondary to craniopharyngiomas, large, slow waves were recorded from the surface of the distended dura overlying the middle frontal convolution. Following a ventricular tap by means of a brain cannula, the slow waves disappeared immediately and were supplanted by beta activity (Case IX, Fig. 12). The rapidity with which this change took place was startling.

There seemed, however, to be some variability in regard to the presence of the slow waves with the increased intracranial pressure. One of our cases was re-operated upon ten days later with a recurrence of the hydrocephalus and intracranial pressure. This time, despite a similar degree of dural distension, slow waves were only occasionally present. This finding appears to corroborate the findings of Williams (14), showing that there was no direct, or at least constant, relation between the development of increased intracranial pressure as such and appearance of slow potentials.

(E) *Electric potentials associated with focal trauma to the brain* were studied in one patient (Case X), who had a cortical scar resulting from an old depressed fracture of the skull of five years' standing. In this instance large, slow waves were obtained from the immediate vicinity of the scar tissue (Fig. 13).

(F) *Diffuse pathological diseases of the brain*, as might be expected, showed normal brain rhythms interspersed with slow waves over the entire area exposed. The differences between the electro-cortical dysfunction produced by the atrophy of arteriosclerosis (Case XI, Fig. 14A and B) and diffuse encephalopathy (Case XII, Fig. 15B) seems to be one of degree rather than of quality. There appears no characteristic change which would enable one to differentiate further in this group. The degree of electro-cortical dysfunction is roughly proportional to the extent of the pathological process.

(G) *Electro-cortical activity in association with focal clinical convulsions* was studied in four cases. All of these patients manifested sharply focal motor seizures affecting primarily a single extremity, and being confined to one side of the body. In all cases tumor of the brain and other gross cortical lesions had been first excluded by means of pneumo-encephalograms.

Widespread and diffuse electro-cortical dysfunction was revealed in the electro-corticograms taken from the exposed brains of each of these patients, in spite of the fact that the clinical manifestations in each instance were exquisitely focal (Case XIII, Fig. 16 and 17; Case XIV, Fig. 18 and 19; and Case XV, Fig. 20 and 21).

Maximum abnormality, on the other hand, did appear in each case to come from a region corresponding generally to the site of the "epileptic focus," as pre-determined upon clinical and anatomical grounds. This is well shown, for instance, in Case XIV, Fig. 18 and 19, where it is evident that the abnormal activity coming from point No. 10 is greater than that coming from other parts of the cortex. It is also clearly demonstrated in Case XV, Fig. 20 and 21. Here it is evident that the most marked electro-cortical dysfunction arises from the region of markers 8 and 9, which correspond closely to the presumed epileptogenic focus, although it is manifest that the electro-cortical activity of the entire hemisphere is abnormal.

The type of abnormal electrical activity found in these convulsive disorders consisted entirely of large, slow potentials of 3-5 cps., generally indistinguishable from the abnormal potentials associated with tumors. No "spike" formations were seen (Figs. 17-19 and 21).

The evidence contained in these four cases of focal convulsions suggests that the focal phenomena in such cases may be merely an expression of *maximum* abnormality coming from a cerebral hemisphere, which, as a whole, is functioning in an abnormal manner. An epileptogenic "focus" might be regarded, therefore, merely as an area of maximum abnormality in a brain affected by a diffuse electro-cortical dysfunction.

IV. DISCUSSION

The central sulcus appears to be the most significant anatomical boundary for spontaneous rhythmic activity. The alpha rhythm or rhythms arise almost solely in the parietal, temporal and occipital lobes. Likewise, the beta rhythm or rhythms occur most prominently in the frontal lobes, though they may appear with greatly diminished amplitude elsewhere in the cortex. The central sulcus limits the alpha rhythm more definitely than it does the beta activity.

It might be said that the alpha rhythm seems to be allied in a very general way with sensory function. This concept is supported by the fact that all forms of sensory stimulation are capable of abolishing the alpha rhythm, a finding which would be difficult to explain if the alpha activity arose only in the optic cortex, as some authors have suggested.

Beta activity seems in the same general way to be associated with motor function. In one case of focal convulsive disorder, the beta potentials in the portion of the motor gyrus which represented, upon stimulation, the anatomical focus of this disorder, showed spasmodic prolonged outbursts of very high amplitude.

In one case it was possible to put electrodes directly on the optic nerve.

Rhythmic potentials of alpha frequency were found to be present. Gerard (15) has reported that in the cat the spontaneous rhythm found in the optic cortex is present throughout the entire optic tract. *It is both interesting and significant that these rhythmic potentials should be transmitted over fiber tracts. It is possible that this type of fiber tract transmission is responsible for the foci of abnormal activity which commonly appear over the frontal lobes with cerebellar tumors (16).*

The abnormal potentials which are present in the tissue surrounding a benign encapsulated tumor are probably caused by mechanical deformation of the cortex. This is shown by the fact that abnormal potentials are found only in the immediate vicinity of the tumor. Likewise, the relative paucity of abnormal potentials associated with this type of tumor suggests that although it may have greatly deformed the cortex, the change has been slow enough to allow the surrounding neural tissue to make adequate physiological adjustment.

With the infiltrating type of tumor the area of damage is large and the physiological impairment ranges from a lowering of the frequency of the normal rhythms, to complete absence of rhythmic activity. These findings seem to fit a pathological picture varying from mild cellular edema to completely degenerated tissue.

The findings in hydrocephalus are variable and difficult to understand. Apparently under certain conditions it is possible to produce generalized abnormal potentials which are rapidly reversible with the release of the increased pressure. This finding has also been demonstrated by Williams (14). The only fact that appears clearly is that the production of abnormal potentials is not related to the manometric pressure alone.

In the four patients studied, who, clinically, had sharply focalized convulsions, it was found that abnormal electrical activity was found to be *widespread throughout the hemisphere. On the other hand, the region from which the most abnormal electro-cortical activity arose did correspond roughly to the site of the so-called epileptogenic focus, as determined upon clinical and anatomical grounds, and by electrical stimulation.*

These few observations, therefore, suggest that a so-called epileptogenic focus may represent merely the point of maximum abnormality in a brain affected with diffuse, widespread electro-cortical dysfunction. Such a concept, of course, if sustained might lead us to revise our present thoughts relative to the surgical attack upon epilepsy. However, this subject requires much more data and study before the truth can be known.

In several cases it was necessary to deepen the narcosis by means of inhalation ether anesthesia. It was observed that the ether shortly abolished the normal spontaneous rhythms and produced large slow waves in the order *of one cycle per second or less. It is quite necessary to take into consideration, therefore, both the type of anesthetic used, as well as the depth of anesthesia if the spontaneous cortical activity is to be evaluated. Our own ob-*

servations did not justify us in making at this time any generalizations on this subject.

V. SUMMARY

A compact, portable apparatus has been described and a simple technique has been outlined which permit reliable records of electrical potentials to be taken directly from the exposed brain at operation.

The regional distribution of normal spontaneous cortical potentials has been outlined. Alpha activity was obtained directly from the exposed optic nerve.

Tumors were demonstrated to be electrically inactive. This was found to be true for meningiomas, gliomas of various types, craniopharyngiomas, and metastatic tumors.

The slow potentials commonly associated with tumors were found to arise from the damaged tissue adjacent to them, rather than from the tumors themselves. The area in which these abnormal potentials occur is small with the benign encapsulated tumors and much greater with the infiltrating type.

Changes in electrical potentials in association with hydrocephalus, cortical cicatrices, cerebral atrophy secondary to arteriosclerosis, and diffuse encephalopathy have been reported.

Abnormally large, slow potentials were obtained from widely distributed points of the cortex in patients who, clinically, exhibited sharply focal convulsions. This dysfunction appeared greatest at points which corresponded roughly with an epileptogenic focus, as determined on anatomical and clinical grounds. The electro-cortical dysfunction seemed definitely greater in the pre-central areas of the brain than it did in the post-central regions. "Spike" formations were not observed.

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EFFECT OF CHRONIC PAINFUL LESIONS ON DORSAL ROOT REFLEXES IN THE DOG*

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THE CLINICAL observations of Leriche (1939), Livingston (1938, 1940), and others in cases of "pathological" pain syndromes such as causalgia, etc., have been difficult to explain on the basis of accepted concepts of neurophysiology. The persistence of the pain after section of peripheral nerves and dorsal roots and occasional relief for long periods following novocaine injection of an irritative focus or "trigger point" has led one of us (W.K.L.) to postulate that long continued irritation of a sensory nerve might modify the normal physiology of the central nervous system.

The dorsal root reflexes described by Toennies (1938a) might be related to these pain syndromes. The fact that these reflexes are accentuated under certain abnormal conditions, namely decreased body temperature, Barron and Matthews (1939) and Toennies (1939), suggested the possibility of a relationship between dorsal root reflexes and these pain syndromes which are apparently "pathological," i.e., abnormal reactions in certain individuals to irritative lesions.

METHOD

The presence or absence of reflex discharge of fibers in two sensory nerves, the lateral branch of the dorsal radial cutaneous and the saphenous nerves, following single electric shocks applied to the same nerves, was determined in 17 apparently normal dogs. In 8 of these at the time of the first observation two drops of croton oil were injected into the nerve sheath of each nerve. After a period of 21 to 25 days, while the ulcers formed were unhealed and apparently painful, the same nerves on the opposite side were likewise tested for a reflex discharge. In 8 additional dogs with a single painful ulcer of long standing (15 to 40 days) the remaining three nerves were similarly tested. All records were made at normal body temperature of 39.2°C. rectal (no records of spinal temperature were taken). At the conclusion of each experiment observations were made on the effect of lowering the body temperature on the reflexes.†

The animals were lightly anaesthetized with Nembutal, 1 cc. of a 6 per cent solution per 11 kg. body weight. At the level of anaesthesia used the tendon reflexes were always readily obtainable. The nerves were dissected free, cut peripherally and stimulated and led from as indicated in Fig. 1a. A few records were taken with similar leads placed on the dorsal roots.

Monophasic records of both a primary action potential conducted peripherally from the stimulating electrodes as well as the reflex discharge which it produced were obtained. The percentage fiber response was based on the area of the first spike of the reflex discharge compared to the total area of the maximum response of the fibers of fastest conduction (A fibers) as measured from the primary action potential following correction for differences

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† In the first few experiments an ordinary electric heating pad was used to keep the
1. The alternating current (60 cycle 110 v) induced a
when led to ground through the nerve was of sufficient
cord to be conditioned and no reflexes were obtained
for as long as 10 sec. after the electrical pad was turned off preparatory to taking records.
In the remaining experiments the animals were warmed by a hot water heater.

in amplification necessary to record the two different potentials. A cathode ray oscillograph and differential amplifier, Toennies (1938), was used.

RESULTS

Of the 17 animals with no lesions, 8 showed dorsal root reflexes at normal body temperature and there were no reflexes in 9. Of the 16 animals with lesions, 7 showed reflexes and 9 did not.

In the 8 dogs in which tests were made both before and after lesions, 5 showed reflexes before the lesions and 3 did not. After the lesions, 2 of the animals which had reflexes failed to show them, while 2 of the animals which had previously failed, now gave reflexes.

The size of the reflexes was no different in the animals with lesions than those without lesions.

In the typical oscillogram, Fig. 1b., the first spike to appear is the action potential in A fibers stimulated electrically and conducted peripherally. The reflex spike appeared 10 to 15 msec. later and in many of the experiments a second reflex spike was seen. It began 18 to 23 msec. following the stimulation. At normal body temperature the first reflex spike usually involved 3 to 5 per cent of the total A fibers. However, in one experiment 9 per cent were estimated to be involved. The reflex was conducted in the dorsal radial cutaneous nerve at 47 to 52 m. per sec. as compared to a rate of con-

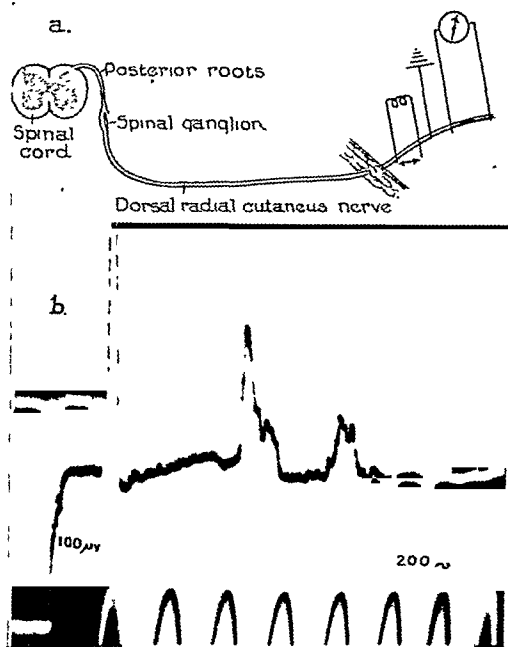


FIG. 1a. Arrangement of the electrodes in stimulating and leading from the nerves.

FIG. 1b. Typical oscillogram. The first spike (incompletely shown because of high gain) is the primary action potential. The reflex response consists of two separate spikes (see text).

duction of 60 m. per sec. for the fastest fiber in that nerve under the conditions of temperature, etc., for these particular experiments. The second reflex spike, when present, was almost always smaller than the first in area. Its conduction rate, though measured with more difficulty, was approximately the same as the first reflex and did not represent activity in fibers of significantly lower conduction rates such as the reflexes in delta fibers described by Toennies (1938) in the cat.

It was confirmed in the dog, as Barron and Matthews (1939) and Toennies (1939) have shown in the cat, that lowering the body temperature increased the percentage of fibers involved in the reflex or brought a reflex into

view which had not been observed at normal temperature. The optimum body temperature was found to be 36.5°C. Under these conditions reflexes were seen which involved 10 per cent of the total A fibers. In no case in the dog, even on cooling, were reflexes observed which approached the size (65 per cent of A fibers) recorded in the cat by Toennies.

The reflexes were more constant and larger in the dorsal radial cutaneous nerve than in the saphenous nerve. A dorsal root reflex was obtained in every animal except one when the body temperature was lowered.

CONCLUSION

It appears that the presence of a painful lesion of 15 to 40 days' duration involving a sensory nerve has no influence on the presence or absence of dorsal root reflexes in the dog.

The work of Toennies (1938, 1939) on dorsal root reflexes in the cat was confirmed in the dog. Reflexes were found at normal temperature in about 50 per cent of the animals studied. The reflexes could be produced or increased in size by lowering the body temperature, confirming the results of Barron and Matthews (1939) and Toennies (1939).

* * * * *

We express our appreciation for technical assistance to Mr. Fred Claussen.

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REFLEX ACTIVITY IN THE SPINAL EXTENSORS

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INTRODUCTION

SINCE Adrian and Bronk (1, 2) demonstrated muscle action potentials from single motor units there have been numerous reports of action potentials accompanying artificially stimulated muscle, voluntarily contracted muscle, muscle tension in neuromuscular diseases, and muscle tension occurring in the performance of imaginary tasks (3, 7, 8, 11, 12). In addition, Jacobsen (6) has reported extensive studies of muscle action currents in nervous and "neurotic" patients.

Except for Jacobsen's studies of "neuromuscular hypertonus" and the material which deals with organic neuromuscular diseases, observations of muscle action currents have dealt primarily with artificially stimulated or voluntarily contracted muscle. A few reports indicate an absence of muscle potentials in normal, relaxed muscle. Lindsley (7) stated, "no electrical activity has been demonstrated in any part of a relaxed muscle," on the basis of records, on 6 normal adult subjects, from the deltoid, biceps, flexor digitorum sublimis, branchioradialis, rectus femoris, vastus lateralis, sartorius, gastrocnemius and tibialis anticus. Smith (12) also observed no electrical activity in the relaxed biceps and triceps.

Osteopaths and many orthopedic surgeons report the clinical finding of increased resistance to pressure deformation of the periarticular tissues of spinal, sacro-iliac and appendicular joints in certain cases which manifest neither obvious organic pathology nor voluntary muscle contraction.

In the present study, an attempt has been made, by electromyographic findings, to determine if reflex muscle activity underlies in part these clinical findings.

METHODS

In a majority of instances concentric needle electrodes (22 gauge) were used. Occasionally two plain hypodermic needles, a hypodermic needle and a skin pad electrode, or two skin pad electrodes were used to increase the amount of tissue between leads.

Dual, 4 stage, resistance-coupled, balanced amplifiers drove either loud speakers, a Sanborn cardioscope for visualization, or Westinghouse sensitive bifilar oscillographs for recording on EKG paper. Calibrations were obtained by placing a 10,000 Ω resistor across the leads of one channel and delivering a known input of 10 μ V across a 1 Ω resistance to the other (Fig. 1). Both channels were checked by this procedure.

The subject (in a shielded room) was placed prone on an upholstered table, which had a padded slot, 3 in. wide and 6 in. long, for the face. This permitted the head to be in the mid-line position.

Areas of "lesion" and control areas were selected by palpation of the back and electrodes inserted into the erector spinae mass. (Electrodes and skin were treated with 70 per cent alcohol, and the skin was anesthetized by intradermal injection of 1-2 cc. of 2 per cent procaine hydrochloride.) Voluntary contraction, involuntary tension and respiration of the thoracic type are all accompanied by potentials in these muscles (Fig. 2). Hence,

studies of postural or attitudinal reflex activity must be made with the subject completely relaxed and during the few seconds between exhalation and inhalation.

In 6 experiments, after recording from the lesion and normal areas, two additional symmetrical areas, normal on palpation, were studied under comparable conditions.

Subjects

Normal relaxed muscle is soft and resilient; it is not tender to moderate pressure. In the thoracic region the extensor muscle masses can be palpated as columns or segments

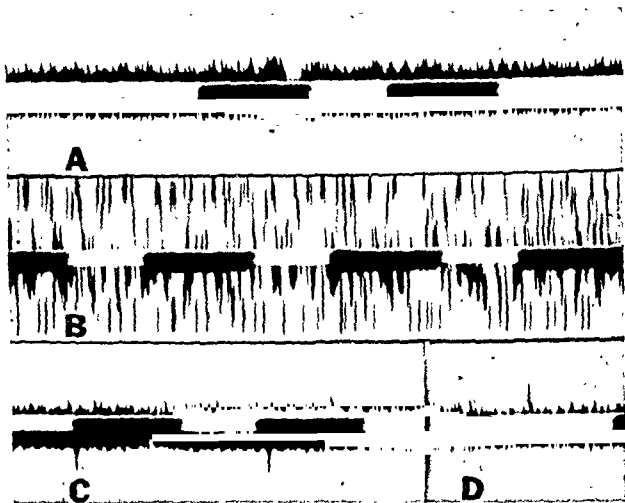


FIG. 1. Upper and lower tracings—concentric electrodes in two normal areas. (A) Subject relaxed at end of exhalation, the single blunt spike is the cardiac artefact. (B) Mild voluntary contraction of the thoracic extensors to demonstrate that both electrodes were in muscle tissue. This control (B) was used in all experiments. (C) Upper tracing—10,000 Ω resistor across input. Lower tracing—input of 10 μ V from dry cell and voltage divider. (D) Reverse of (C). Time signal—0.25 sec.

which become firmer on voluntary contraction. Muscle in a "lesion" segment is abnormally firm; it is less resilient and has a characteristic texture which simulates local spasm or contraction even in the relaxed subject. The muscle columns feel indurated and ropy and roll under the fingers. There is abnormal tenderness and deep pressure may cause considerable pain. Such lesion areas are frequently unilateral and limited to one or two spinal segments.

Twenty-five records were taken on 16 students and instructors in whom areas of abnormal tissue resistance were found by palpation. All were free from gross disease, except for one with mild attacks of asthma, and carrying full work. Each one, however, had a postural error in the form of either a slight lateral curvature or an obliteration or an exaggeration of the normal anatomical curves. Some subjects gave a history of having had discomfort in the "lesion" area, others not.

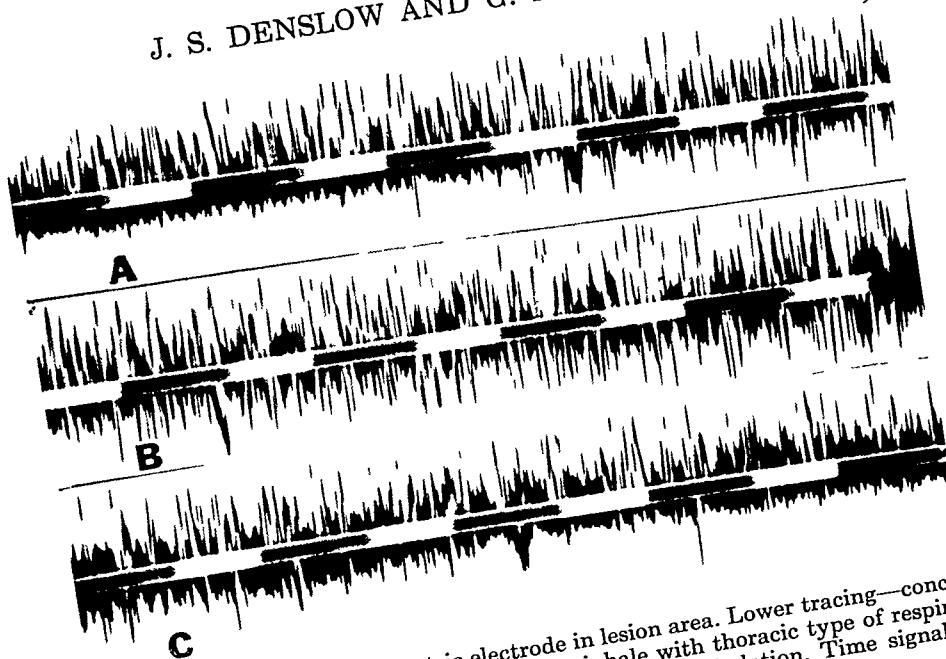


FIG. 2. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Subject starting to inhale with thoracic type of respiration; (B) Subject at height of inhalation; (C) Subject at end of exhalation. Time signal—0.25 sec.

RESULTS

Localized reflex motor unit activity was seen in a lesion area in 21 of the 25 experiments in this series. That this activity was local, and not merely a part of a generalized neuromuscular hypertonus is shown by the absence of activity in an adjacent normal area observed simultaneously.*

The reflex activity recorded varied in degree during each experiment, from occasional quiet periods to activity of single units or of many (Fig. 3). There was no definite pattern to the rise and fall of activity except that the quiet periods were more frequent when the subject first reclined, activity increasing as the subject became fatigued from being in the same position for 30 min. or more. Both the number of active units and the frequency of single units were increased by respiration of the thoracic type, by mild voluntary contraction, and by involuntary "tension."

Single active units were active at frequencies from 3 per second to 24 per sec., but in 23 records, of 29, the frequency was between 6 and 10. While some frequency change occurred without apparent cause (e.g. from 10 to 24, B in Fig. 3), the frequency of a single unit was invariably increased by each of the factors listed above.

Inhibition of single units

In individual instances a single active unit in the lesion area could be inhibited.

* In earlier work, with only one channel available, an equivalent observation was made many times by rapid switching over from one area to the other.

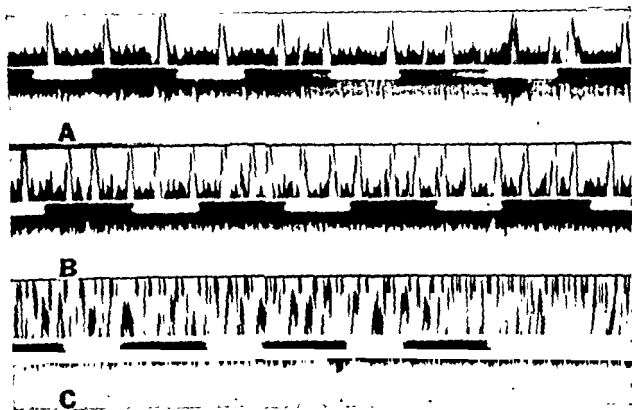


FIG. 3. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Single active unit in lesion area; (B) Either an increase in rate of unit active in (A) or an additional active unit; (C) Numerous units active in lesion area. Time signal—0.25 sec.

hibited by (i) extremely slight voluntary contraction of the abdominal wall, (ii) shallow respiration of the abdominal type, (iii) counting or talking aloud (Fig. 4) and (iv) by swallowing.

Several factors are necessary for the successful inhibition of the reflex activity of a single unit. Most important is the strength or persistence of the active reflex. In some instances this seemed to be "borderline" and the activity came in and faded out. Slight changes in position and momentary periods of voluntary contraction might then result in a cessation of activity. Or, the area might be quiet until activity was initiated by a slight movement of the skin about the electrode.*

Inhibition produced by flexion occurred in these borderline areas, not where the lesion reflex persisted without periods of inactivity.

Additional factors which mitigate against demonstrating flexor inhibition are the presence of more than one or two active units, and the inability of the subject to limit voluntary effort to the flexor groups exclusively but who became tense when trying to follow instructions.

* McKinley stated (10), "As to the movements of electrodes producing action currents, no doubt that happens, and yet we have been able to place our electrodes in the muscles and then massage those muscles in rather lively fashion without any action potentials being developed whatsoever."

We have had the same experience. When the electrodes are in normal muscle the skin about them may be moved to a considerable degree without initiating action potentials

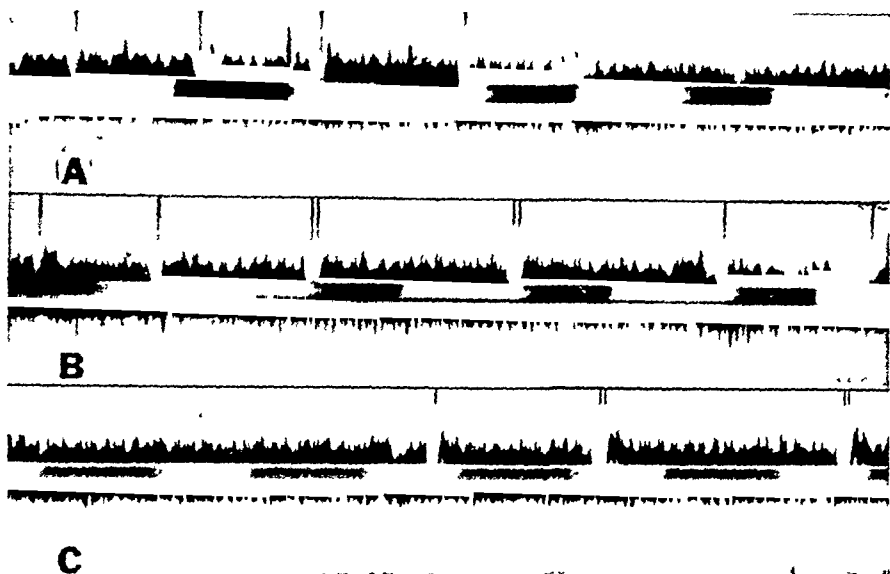


FIG. 4. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Subject inhibited active unit in lesion area by slight contraction of anterior abdominal muscles; (B) Subject completely relaxed; (C) Subject had inhibited active unit by counting aloud, the record shows the unit coming back in. We have been unable to determine if the double spikes in (B) and (C) represent two active units or a peculiar double spike from one. Time signal—0.25 sec.

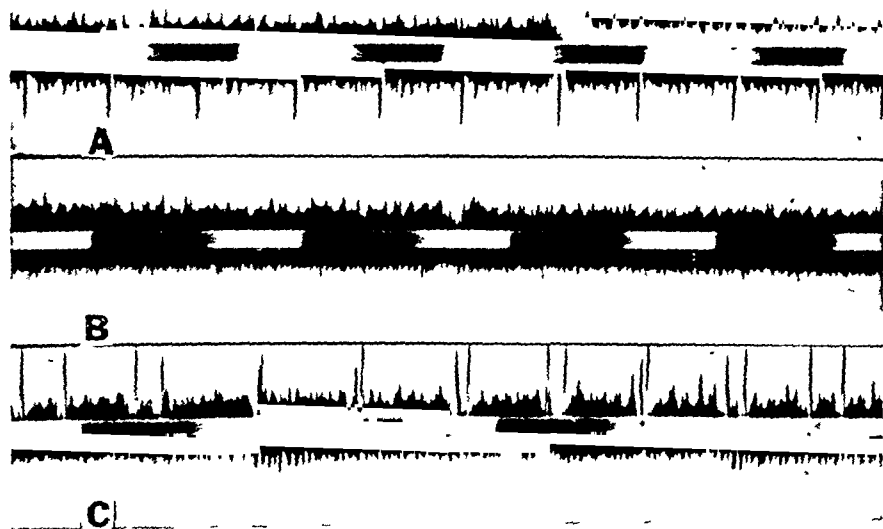


FIG. 5. This record is the first portion of the experiment shown in Fig. 4. The electrode of the upper tracings is in a lesion area, the one in the lower tracings is in a normal area. The active unit of the lesion area had not started to fire when this part of the record was taken. The upper tracing is of the right side, the lower of the left. (A) Subject's head turned toward the left; (B) Head in mid-line position; (C) Head turned toward the right. Time signal—0.25 sec.

Absence of activity in lesion areas

In four experiments no activity was observed in the lesion area. In each of these the observations were made over 30 to 60 min. and the electrodes were slowly removed from the muscle and reinserted at least six times, to insure against missing a small localized area of activity.

Postural reflexes

In the first studies the subject was placed prone on the table with the head turned to one side. Inconstantly, and never in some subjects, varying degrees of activity were seen in the thoracic extensors on the "jaw" side while the "vertex" side was quiet (Fig. 5). Sometimes, when the neck reflexes were not present with the head turned to the degree required by the prone position, activity on the jaw side appeared when the operator forcibly rotated the head farther, or lateral flexed the head toward the shoulder on the jaw side. In other cases forcing the head even to the point of discomfort did not produce activity.

DISCUSSION

The motor unit activity demonstrated in lesion areas is local and is reflex in character. It is similar in frequency, in asynchronism and in recruitment to the stretch reflex as described by Creed, Denny-Brown, Eccles, Liddell and Sherrington (4) with the exception of the finding of low frequency rates in 10 records. There is also similarity with the extensor response in the postural reflex as first described by Magnus (5).

The action potential findings in the lesion area have characteristics identical with the stretch reflex in their response to various tensions and to head position. However, they were observed when the subject was completely relaxed, in the "resting" period of the respiratory cycle and with the head in the mid-line. The activity occurred in local areas where palpation revealed apparent abnormality in the tissue. Hence, they represent a muscle contraction which could be designated as an abnormal reflex.

The difference between the stretch and postural reflexes and the lesion reflex lies in the cause of its development; there being no stretch or positional asymmetry in the latter. The cause is either a stimulation of tension receptors within the muscle itself or stimulation of other receptors which are segmentally related.

Clinical observations have led to a general acceptance of viscerosomatic reflexes, such as the right rectus muscle contraction in cases of acute appendicitis, yet neither biochemical nor bio-electrical studies of these types of "functional pathology" have been made. It is possible that the lesion reflex might be viscerogenic in character. However, this does not seem probable as none of the subjects, with the exception of one asthmatic, was suffering from gross visceral disease.

A more logical explanation is found in the fact that all subjects presented postural abnormalities of various types. As the articular capsules and the

tendon and ligamentous supports of the spinal articulations are richly supplied with proprioceptor end organs, the abnormal pressures and tensions developed in functionally pathologic joints might well cause the lesion reflex. Steindler (13) points out that "under pathologic conditions, however, we may find the spine unprotected against the fluctuations in gravital or dynamic stresses, and strains or even fracture may result." Under these pathological conditions the spinal cord is abnormally bombarded with afferent impulses from the area and a reflex discharge is maintained.

Joints which show some arthritic changes become "stiff" and uncomfortable when the patient reclines for an hour or more. They improve when he moves about. This is probably a parallel to our observation that at times motor unit activity in the lesion area is not seen until the spinal articulations have been relatively immobile for some period of time.

In some experiments slight movement of the skin around the electrode in the lesion area was followed by motor unit activity while the normal control areas, similarly treated, remained quiet. Here the lesion reflex perhaps created an enduring subliminal central excitatory state in a motoneuron pool (5). Additional stimuli from electrode movement, themselves subliminal, can then cause excitation on reaching the motoneuron pool. Conversely, activity of the lesion reflex was temporarily terminated by flexor activity, only when the neurons bombarded from the lesion area were apparently just above the threshold of discharge and could be inhibited by the prepotent flexor reflex.

There are several possible reasons why, in several experiments, no motor unit activity was found in lesion areas. The most likely is that there was none present at that time and that the apparent tenseness in the muscle was due to some factor other than muscle contraction. That activity existed but was not located does not seem likely, as several samplings of the muscle were made by repeated reinsertions of the electrode in each instance. Another possibility is that the presence of the electrodes inhibited activity. Such a phenomenon was surmised by Davis (in discussion of McKinley and Berkwitz, 10) and we have sometimes noted that a lesion area felt softer after the needle was removed. The fact that in some lesion areas there were periods of activity and of inactivity suggest a final possibility that the lesion reflex might be phasic and that when electrical activity was not found it was because the examination was made during a quiet phase.

SUMMARY

1. Reflex muscle activity, similar to stretch or postural reflexes, is seen in areas of "lesion" in the erector spinae muscles when the subject is relaxed, the extremities in symmetrical position, and the head and face in the midline. Adjacent normal areas do not show motor unit activity. One or many motor units may be active, sometimes after a delay of 4 to 45 minutes after inserting the electrodes.

2. Single units discharge at rates of 3 to 24 per second.

3. A single active unit firing intermittently in the lesion area could at times be stopped by flexor contraction.
4. In a few instances motor unit activity was not seen in an area of lesion.
5. In some normal subjects muscle activity appeared in the spinal extensors on the "jaw" side when the head was turned.

We are indebted to Drs. R. W. Gerard and C. Ladd Prosser for their encouragement, suggestions and advice.

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STEADY POTENTIAL FIELDS AND NEURONE ACTIVITY*

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THE ISOLATED FROG BRAIN continues to manifest essentially stationary rhythmic potential oscillations and, under the influence of drugs such as caffeine, slowly traveling potential waves (Libet and Gerard, 1939; Gerard and Libet, 1939, 1940; Gerard, 1941). Such phenomena demand, respectively, the synchronous or successive electrical discharge of large numbers of neurones. Experiments with tiny bits of olfactory bulb, with changed intercellular ionic milieu, with added drugs, with altered temperature, etc., demonstrated that the mechanisms controlling the beat of the individual neurone and the set timing of many neurones are not restricted to the familiar conduction of impulses along neural paths.

The case is especially clear with the large caffeine waves, which travel at about 6 cm. per sec. mainly from the anterior to the posterior pole of the cerebral hemispheres; for these waves are not abolished by nicotine, which blocks synaptic transmission (Libet and Gerard, 1938; Schweitzer and Wright, 1938), and often not even by a sharp complete transection of the entire cerebrum. Since a traveling wave can cross a cut in its path, nerve conduction cannot be responsible; and since a slight separation of the cut halves, even with a film of Ringer connecting them, does block the wave, a chemical mechanism is probably excluded. There remains the possibility of electric currents, flowing through intercellular fluids, as a basis of neurone interaction.

An hypothesis was developed (Gerard and Libet, 1940) which explains the propagation of electric waves along the brain in a manner analogous to the propagation of an impulse along a nerve fiber. The neurones in the frog's hemisphere are in the main oriented alike, with their dendrites directed toward the pial surface and their axones emerging from the ventricular pole of the cell body; and, further, the cell bodies are gathered into a sheet, a few cells thick, paralleling these gross hemisphere surfaces (Fig. 1a. See also Cajal, 1911, p. 844; and Herrick, 1927, Fig. 6 to 9). Assuming that the soma of each neurone is polarized from dendritic to axonic poles (the somatic potential), a D.C. potential maintained by the cell's active metabolism, the whole cell sheet would behave like the polarized membrane of a nerve fiber. A local depolarization, resulting from the discharge of one cell or a few adjacent ones, would permit neighboring cells to discharge through the "leak" and so initiate a spreading wave of depolarization (see Gerard and Libet, 1940, Fig. 16). The "action current," flowing through conducting fluids—

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(Left) A.* Photograph of cross-section through cerebral hemispheres, *Rana catesbeiana*, about 1 mm. caudad to the junction with the olfactory bulbs, stained with toluidine blue for cells. The position of the PV electrodes is indicated. Enlargement 10 X.



B. Diagram showing usual position of PV and S_1, S_2 electrodes on a longitudinal section of the brain.

FIGURE 1

"horizontally" superficial and deep to the cell layer, and "vertically" between cells and to some extent through the cell bodies—would give the recorded traveling potential wave and would be the means of propagated excitation.

There are some hundred neurones per linear centimeter. To account for the observed rate of travel, a detonator delay (Lorente de Nó, 1935, Eccles, 1936) of 2 msec. at each cell would be required—not an unreasonable figure for cold blooded brain. Also, the caffeine waves tend to an all-or-none character—they usually invade a region suddenly and at full size and maintain their size and rate even when greatly decreased in frequency (Fig. 2A)—although their magnitude can be varied.

Such an interpretation predicts the existence—and direction—of a constant potential gradient between pial and ventricular surfaces; and it makes specific predictions concerning the influence of polarization through or along the hemisphere wall upon the spontaneous waves and those induced by caffeine. These we have investigated.

METHODS

Fluctuating potentials were recorded with Ag-AgCl wick electrodes, 0.2 to 0.8 mm. diameter, shielded nearly to the tip. Two pairs fed independent channels and crystalographs, and controls demonstrated the absence of interaction. Polarizing currents were applied also through such electrodes, sometimes with continuing recording of the brain potentials. For measuring D.C. potentials, the more stable Zn-ZnSO₄-Agar-wick electrodes were always used, and these sometimes served as well for recording the A.C. potentials simultaneously or for polarizing.

When one electrode was to lead from the ventricle, its wick dipped into the open end of a pipette, filled with Ringer's solution and held horizontally, the narrow (1 mm.) bevelled tip of which was inserted into the ventricle so that the bevel faced the paired electrode on the dorso-lateral pial surface of the hemisphere (Fig. 1). (The pipette tip can be seen

* We are indebted to Dr. O. Sugar for preparation of these sections.

through the thin hemisphere wall, and the pial electrode placed accurately over it.) The ventricle was opened by transecting the hemispheres with a sharp razor near the posterior pole; and the pipette could be inserted to about 2 mm. caudal to the olfactory bulb (Fig. 1b), while the cut medial and lateral walls were gently separated, without its tip producing injury. When the walls were then allowed to collapse, the pipette was "sealed" in place.

D.C. potentials were measured by a balancing potentiometer on a sensitive galvanometer. The high resistance circuit limited current drain from the brain to 10^{-7} A., and this only during the instant that the circuit was closed while attaining balance. Later, even this minimal current was eliminated by using a one-stage high input-impedance D.C. amplifier.* Potentials could be read accurately to 0.01 mV, but the electrode instability was some ten times as great.

Caffeine was applied in 0.5 per cent solution, in which the brain was usually soaked for 0.5 to 2 min.

STEADY POTENTIALS

Since the olfactory and other cranial nerves and the medulla are cut in removing the brain, and the hemisphere is sectioned for placing a ventricular electrode, the presence of injury potentials must be considered. Direct measurements, between a freshly cut surface and an uninjured region or the surrounding Ringer's solution film, showed that the initial injury potential of 5 to 10 mV normally falls to less than 1 mV within 10 min. Further, the injury potential in the brain is localized to within 1 mm. of the cut surface, for an electrode placed on the surface 1 mm. from the cut edge is isopotential with one still further away. In the following experiments both electrodes were several millimeters from the injured region and often equidistant from it, so that the potentials recorded were not the result of injury. Further, an electrode in the ventricle commonly showed this to be positive to the outer brain surface. Since any injury produced by inserting the pipette should tend to make the ventricle negative, such a source of error can also be excluded.

The uninjured surface of the cerebrum is not, however, at a uniform potential. Most commonly a regular antero-posterior gradient is present, with the olfactory bulb 0.5 to 2 mV negative to the occipital pole of the hemisphere. A similar voltage gradient in the medio-lateral axis makes the midline up to 1 mV negative to the lateral surface. A fall in temperature decreases these potential differences. As described, both gradients show a progressive, and like, potential rise with increasing electrode separation. There are, however, not infrequent exceptions. Rarely, potential gradients have been encountered of as much as 20 mV on the longitudinal axis and 15 mV on the transverse one. Rather common is the presence of a sharp potential change—as much as 5 mV in 1 mm.—at the junction of olfactory bulb and hemisphere. The bulb may also be positive rather than negative to the hemisphere, and the gradient in the hemisphere may itself be reversed. In fact, the center of positivity or negativity may slowly wander about a single quiescent brain over a period of minutes. (The magnitude of the longitudinal steady potential is not related to the frequency, amplitude or smoothness of spontaneous waves, except that both tend to diminish and disappear together.)

* We are indebted to Mr. G. R. Carlson for building this amplifier and to him and Dr. Helen B. Carlson for assisting in experiments with it.

This lability is distinctly increased by caffeine, the potential between two electrodes changing amount and even direction during a reading. In one case, a reversal and swing of 11 mV occurred within a few min. Rather consistently, this drug diminishes the anteroposterior gradient and often makes the bulb its positive end. These changes may well be related to the potential across the hemisphere wall and are of significance in interpreting the origin of caffeine waves.

According to the hypothesis outlined above, the somatic potentials of the oriented neurones should lead to a potential difference between pial and ventricular surfaces of the hemisphere wall. Such a pial-ventricular potential is observed; we shall call the observed potential the PV potential, in distinction to the hypothecated somatic potential. The PV potential averages 2 mV, P negative to V. It may reach 10 or more or fall to zero or even reverse by one or two millivolts, but large deviations are rare. In any particular brain, the PV potential is fairly stable, ordinarily varying only by tenths of a millivolt from minute to minute. The rhythmic waves are usually abolished when the hemisphere is sectioned, though the PV potential persists.

Caffeine diminishes the PV potential (as the surface ones) to an average of 1 mV in the early stages of its action and to zero in half an hour. The shift, in fact, commonly continues until P is positive to V (up to 3 mV); and this reversal may occur early. The character of the traveling caffeine waves will be seen to be closely related to the PV potential; and their origin near the junction of bulb and hemisphere may well be related to decrease of the somatic potential especially at this region.

CAFFEINE WAVES

Previous records of the caffeine waves had been made from the brain surface, with mono- or bipolar electrode positions. Records taken across the hemisphere thickness, with pial (P) and ventricular (V) electrodes, should eliminate many complications introduced by travel of the wave past successive surface leads, and should more directly measure somatic potential changes. These expectations are borne out. The waves from two surface electrodes (S_1 and S_2) are more complex than those recorded simultaneously from PV electrodes; and the former may change form (presumably as the path of the waves shifts relative to the electrodes and alters the diphasic relations) while the latter remain unchanged (Fig. 7B, see also Fig. 5). The PV amplitude should also be larger than the S_1S_2 , by hypothesis; and it is so in fact. (The S_1S_2 wave may be reduced also by diphasic interference.)

A typical PV record of a single caffeine wave is shown in Fig. 2B, and of a train of after-waves in Fig. 2C. The wave starts with a surface positive swing (P + to V) which passes over to a more enduring surface negative potential. Sometimes a final positive phase appears, although an amplifier artifact may be involved in this. An inconstant surface negative notch may introduce the first main surface positive wave. (Figure 4d shows this in accentuated degree at the arrow. See also 3b, 5f and g.) Commonly superim-

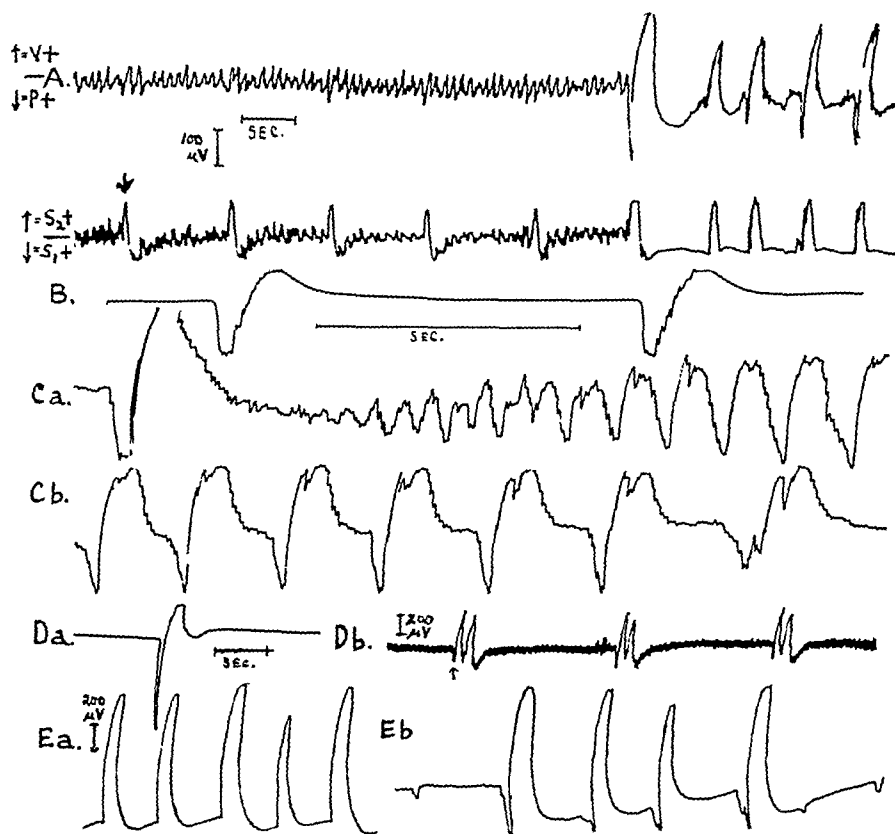


FIG. 2. Caffeine waves. A. Simultaneous PV (top line) and S_1S_2 (bottom line) recording. Caffeinated brain just developing caffeine waves; arrow indicates the first one that appeared. The early waves are originating near S_2 , since this electrode first becomes positive and since no activity is then shown by P, near S_1 . Each caffeine wave appearing in the PV record starts with P+. Note the sudden reduction of the regular spontaneous rhythm with the onset of the "caffeine waves." (Their presence in the early part of the S_1S_2 record is probably from S_1 , which the first caffeine wave does not reach.) Note also that the first caffeine waves appear at full size. The independent records obtained from the two sets of leads are evidence against any electrical interaction between the different pick-ups or amplifying channels.

B. Another brain, caffeine waves, PV recording.

C. Another brain, PV recording. (a) and (b) continuous, except for an interval of 1.7 sec. with one wave.

D. Another brain, (a) caffeine wave, PV recording; (b) during polarization, P+ to V, .01 mA. Arrow indicates surface positive swing; note relatively large size of surface negative waves.

E. Another brain, (a) during polarization, P+ to V, .02 ma.; (b) 10 sec. after cessation of polarization.

A, D, E, are at speed one; B and C at speed three.

Amplification for all is indicated in A, except as otherwise marked.

Note: In this and subsequent figures an up-stroke indicates V+ to P or S_1S_2 to S_1 (S_1 = the anterior, S_2 the posterior lead on the dorsal hemisphere surface). During polarization shunting by the D.C. current circuit cuts the effective amplification to about $\frac{1}{3}$ the value at other times. The amplification is indicated on records taken during polarization between those electrodes requiring correction.

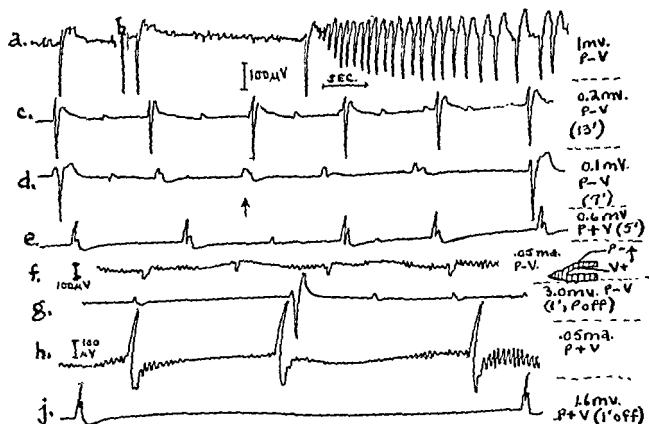


FIG. 3. Caffeine wave changes with D.C. potential (spontaneous and by polarization). PV recording.

- a. Caffeine wave at start.
- b. 4 sec. after a. Measured D.C. potential (PV) 1 mv. P - to V.
- c. 13' later; D.C. potential 0.2 mv., P - to V.
- d. 7' after c; D.C. potential 0.1 mv. P - to V. (Arrow indicates one of the surface negative waves appearing here among the larger surface positive waves.)
- e. 5' later, D.C. potential 0.6 mv., P + to V.
- f. During polarization, P - to V, 0.05 ma. current.
- g. 1' after polarization off; D.C. potential 3.0 mv., P - to V.
- h. During polarization, P + to V, 0.05 ma.
- i. 1' after polarization; D.C. potential 1.6 mv., P + to V.

posed upon the main wave are faster components, at frequencies from 40 to 100 per sec., which perhaps are axon spikes distorted in recording (Fig. 2B and C, 4e). (Discharges at a certain level of potential in the slow depolarization waves of neurones are indicated by the results of Adrian, 1931; Heinbecker, 1936; and Barron and Matthews, 1938.) After-waves resemble in form the initial one (e.g. Fig. 3a, 4a to g).

The time and potential relations of the main wave, while variable with experimental conditions to be considered, and visually distorted by the arched path of the writing pen, are fairly characteristic. In general, the rising limb of a particular wave is briefer than the following fall. The typical surface positive wave attains about 150 μ V and lasts some 130 msec., the negative one is roughly two-thirds this amplitude and essentially twice the duration, although 0.7 sec. may be required for complete return to the base line (Fig. 2B, 4b, 5a). Some voltage loss and distortion of these slow waves are introduced by our amplifiers, of time constant 0.5 sec.

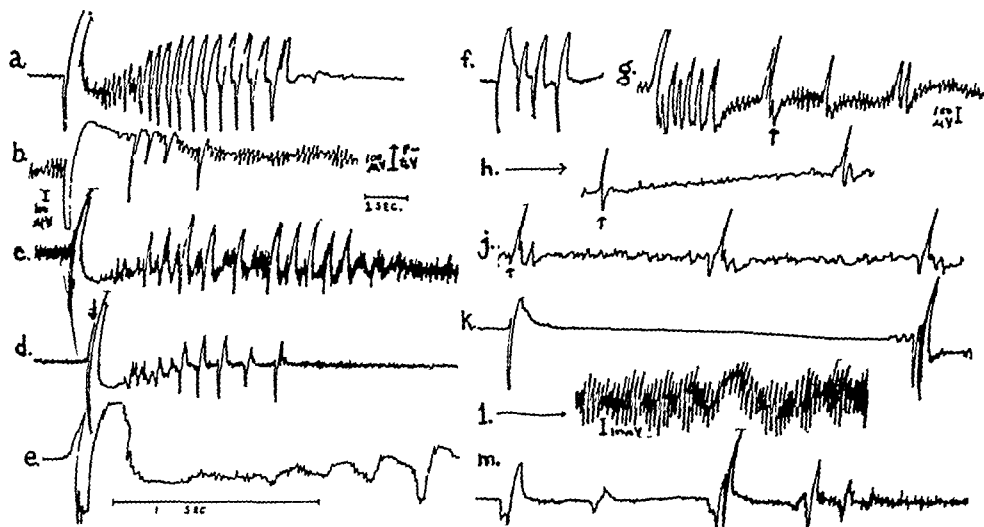


FIG. 4. Effects of pial-ventricular polarization on caffeine waves recorded from same electrodes. All at speed and amplification shown after b, except as otherwise indicated.

- a. Caffeine train.
- b. During polarization, P - to V, .02 ma.
- c. Just after polarization.
- d. Same, 2 min. later; arrow indicates an initial surface negative notch.
- e. Like d, faster record.
- f. 6 min. later.
- g. During polarization, P + to V, .05 ma.
- h. 5 sec. after polarization off; arrow indicates secondary surface positive wave.
- j. Continuation of h (9 sec., without a wave, omitted); arrow indicates initial surface-positive swing.
- k. 3 min. later.
- l. During polarization, P - to V, .05 ma.
- m. 5 sec. after polarization off.

PV POTENTIAL AND CAFFEINE WAVES

If, as hypothesized, the initial surface positive change of a caffeine wave represents depolarization of the existing somatic potential, a close relation should exist between the magnitude and even the direction of the caffeine wave and those of the PV potential. As noted, this latter may spontaneously change, and it is found in fact that the traveling waves alter appropriately. Thus Fig. 3a through e shows a progressive change of the PV potential from the normal, P negative to V, to P positive. The initial positive potential of the simultaneously measured caffeine waves steadily diminishes and finally inverts. (The case of persistent surface positive waves with apparently reversed PV potential, which does occur, will be explained later.) Since the PV potential changes here seen are small and not under control, further studies were made with the aid of constant polarizing currents passed in various directions through the brain.

POLARIZATION

A. Effects on "steady" potentials. A current of 0.05 mA passed for 10 to 30 sec. through the PV electrodes, in contact at their tips, leaves a potential

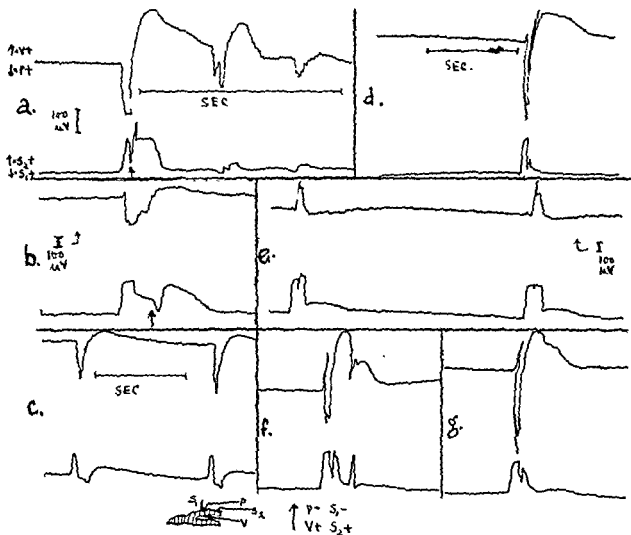


FIG. 5. Effects of pial-ventricular polarization on caffeine waves recorded simultaneously from PV and from surface electrodes. Top line of each pair is PV record, second line S_1S_2 .

- Caffeine wave (amplification is for all records except as indicated).
- During polarization, P^- to V , 0.02 ma.
- 5 sec. after polarization off. (Slower speed for this and following records.)
- 1 min. later.
- During polarization, P^+ to V , 0.02 ma.
- 5 sec. after polarization off.
- 20 sec. later.

difference of about 0.1 mV, in the direction of the applied voltage, which is dissipated within 20 to 30 sec. With the electrodes in position across the hemisphere wall, a similar polarizing current leaves far greater and more enduring potential changes; and the effects have a directional asymmetry.

Thus, 10 sec. after polarization in the direction of the "normal" PV potential (P negative to V) the voltage may be 15 mV. This drops rapidly at first and then more slowly, but even after 6 to 10 min. the potential is 3 to 5 mV and may not return to the original value of 2 mV in another half hour. After a similar polarization in the reverse direction, the immediate voltage may be as great, P now positive to V , but the fall to 3 to 5 mV is achieved in 1 to 4 min.; and by 10 min. the potential has passed through zero to 1 or more mV in the normal direction.

These directional differences are further evidence of a basic polarization—presumably the somatic potential—across the hemisphere wall. That the

retained potential changes after polarization are somehow related to the physiological state (such as the metabolic energy for membrane polarization, the capacity, leakance, etc.) of the cells is evidenced by the altered polarization picture in the caffeinized brain. In this, the post-polarization potential falls rapidly to a steady level, sometimes within one minute and always within five; and this level is essentially the pre-polarization PV potential. Caffeine thus reduces the "polarizability" of neurones as well as their self-generated somatic potential.

Polarizing along the antero-posterior axis also alters the PV potential but to a lesser degree—as would be anticipated from the flow of only part of the current, on the way to deeper tissue regions, perpendicular to the surface. Thus, a current of 0.05 mA passed for 30 sec. from an anterior electrode (S_1) to another (S_2) 6 mm. posterior— S_1 positive to S_2 is a "descending" current—inverts the PV potential measured directly under S_1 from the normal, $P=2$ mV negative to V., to $P=1$ mV positive. A minute or so later the normal potential is reestablished. With "ascending" polarization, S_1 negative to S_2 , the normal PV potential under S_1 is correspondingly increased. Such local changes in PV potential are found to be causally related to the site of origin of traveling caffeine waves.

B. Effects on waves. When the PV potential is altered during, or even for some time after polarization, in the manner just described, corresponding changes are seen in the traveling caffeine waves. Essentially, as the normal magnitude of the PV potential (pia negative) is reduced, the surface positive phase of the wave diminishes and the following negative phase increases; and when the PV potential actually reverses, so does the sign of the wave. In Fig. 3e, for example, the PV potential had spontaneously become P positive to V and the caffeine waves were accordingly also reversed and starting with a surface negative swing. On polarizing with 0.05 mA, P negative, the normal surface positive wave returned and remained until polarization in the opposite direction again reversed both the PV potential and the wave. (Fig. 3f to j. Note that during polarization the recorded wave amplitude is reduced to about one-third, by the shunting of the polarizing circuit. See legend to Fig. 2.)

The effects of polarization can be followed more intimately when even smaller currents, 0.01 or 0.02 mA, are used. When directed so as to increase the normal PV potential, polarization alters the caffeine wave as follows: (i) the surface positive wave increases in amplitude and (ii) doubles or triples in duration; (iii) the following surface negative wave diminishes in size relative to the preceding swing and often on an absolute basis; (iv) the high-frequency superposed waves are suppressed; (v) the secondary surface positive wave, if present, disappears; (vi) fewer after-wave trains follow the initial complex; and (vii) the velocity of the wave transmission apparently decreases in the region of polarization, as judged from the increased delay of appearance of the surface positive notch at an electrode just adjacent to the region of polarization (see arrows, Fig. 5a and b). These changes slowly

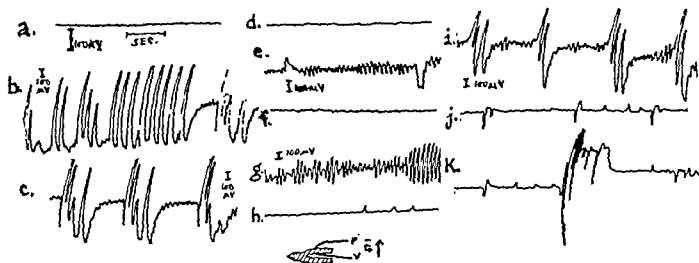


FIG. 6. Initiation of activity in caffeinized brain by polarization. PV recording, except e and f from S_1S_2 .

- a. Silent caffeinized brain.
- b. During polarization, P+ to V, 0.02 ma.
- c. Same, 1 sec. later.
- d. After polarization off.
- e. During polarization S_1 to S_2 , 0.04 ma.
- f. After polarization off.
- g. During polarization, P+ to V, 0.02 ma.
- h. After polarization off.
- i. During polarization, P+ to V, 0.02 ma.
- j. 5 sec. after polarization off.
- k. 3 min. later.

fade during minutes after the polarization is discontinued, except that the amplitude of the initial surface positive wave may sharply fall, to even less than its pre-polarization value (Fig. 5c). (Compare nerve polarization, Bishop and Erlanger, 1926.)

When the polarizing current opposes the "normal" PV potential all these changes occur in the reverse direction; and if the PV potential is sufficiently inverted, the reduced surface positive wave passes over into a surface negative one and is followed by a surface positive wave, which may or may not have been foreshadowed as a secondary surface positivity. With mild polarization, however, the PV potential may be inverted when the waves are not. Polarization may also alter the total incidence of wave trains and single waves, suppress or augment activity; with, commonly, a "rebound" when polarization is stopped. (See Fig. 2D and E; 3f to j; 4, especially b and c; and 5 for examples of these various effects of polarization.)

In Fig. 5 the P electrode (of the PV pair, upper lines) was touching or immediately behind the S_1 electrode (of the surface pair, lower lines). While the PV record is consistent and uncomplicated, the S_1S_2 record can be modified by the position of the electrodes relative to the locus of the wave, the velocity of travel, and the durations of each phase at each electrode. e and hump positive wa.

The rather regular waves at 8 per sec., seen only during polarization—e.g. Fig. 4b and l—are related to vibration of the electrodes, for they are greatly increased for 2 to 10 sec. after a light tap on the floor or the shielding case. (Such a tap in the absence of polarization causes only a brief flurry of faster waves.) That such waves may not be a simple

mechanical artifact is indicated, however, by the following facts: (i) they are greatly reduced when the same procedure is applied to a dead brain, and change with the condition of the living brain, length of isolation, salt medium, etc.; (ii) they are of a frequency close to that of the spontaneous brain waves, and this fixed frequency is not affected appreciably by wide changes in the pendulum or electrical properties of the electrodes; (iii) they outlast, and are of lower frequency than, the electrode vibration—which can be seen as a separate artifact in the record, lasting only a second or two.

Perhaps the slight oscillatory shift, initiated by the electrode vibration, of the polarizing current among the neurones, acts upon them to enhance and synchronize their spontaneous beats. This point must be further studied; for the present, the waves are discounted as artifacts. (A further, high-frequency thermal-noise artifact complicated records taken during polarization until carbon resistors were replaced with wire wound ones.)

C. Effects on the origin of waves. Appropriate polarization is able to initiate activity in silent brains and to shift the site of origin of traveling waves in active ones. The action of ascending and descending currents on the spontaneous beat of the untreated brain has been described (Gerard and Libet, 1940); and no significant restoration of activity has been observed in the transected uncaffeinated brain, which is regularly silent.

Caffeine waves also are abolished by transection in about one-fourth of the brains, and even without section they gradually "peter out"; and these can often be reinitiated by polarization (see Fig. 6 and 7A). A current of 0.01 to 0.02 mA passed through the hemisphere wall (P positive to V), or one of double strength passed between surface electrodes (S_1 positive to S_2 , a descending current), is most effective in initiating activity. In the latter case, waves originate near S_1 , equivalent to P positive (Fig. 7A). Stronger PV currents (0.02 to 0.05 mA) are apparently less effective than the weak ones. Polarization with P negative to V occasionally leads to some activity as a rebound after the constant current is discontinued (Fig. 7Ae and f); but usually if any polarizing current is effective the activity induced by it is maximal early in its passage, may decrease during its continued flow, and outlasts its termination by many minutes (Fig. 6 and 7A).

When caffeine waves are present without polarization, their region of origin is shifted, by a current passed between surface electrodes, so as to approach the positive electrode, i.e., S_1 in descending, S_2 in ascending, polarization. (Polarizing P positive to V seems to have a similar but lesser effect, but this point has not been adequately studied.) A descending current also commonly increases the number and frequency of the caffeine waves, and an ascending current has a depressing effect; as was similarly found for the uncaffeinated brain (Gerard and Libet, 1940).

Evidence for the shift in origin of the waves comes largely from the change of wave forms and time relations as recorded simultaneously from PV electrodes and S_1S_2 electrodes, with P at or near S_1 . Thus, in Fig. 7Ba, the surface wave starts slightly before the PV one and S_2 first becomes positive to S_1 —indicating that the surface positive wave is originating near S_2 . A later dip (arrow) with S_1 positive, probably indicates the arrival of the wave at this electrode. In the course of and immediately after polarization (Fig. 7B, c and d), however, the surface wave starts with or just after the PV wave—indicating that the wave is now originating nearer S_1 . Later (Fig. 7B,

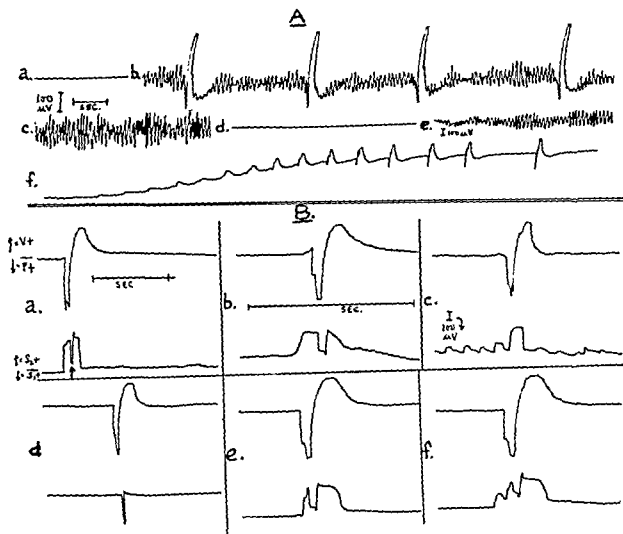


FIG. 7. A. Initiation of activity in caffeinized brain by polarization. All PV recording at speed shown in a. All records in A and B at amplification shown under Aa, except as otherwise indicated.

- Inactive caffeinized brain.
 - During polarization along surface, $S_1 +$ to S_2 , 0.03 ma. (These large waves decrease during the remaining 30 sec. of polarization.)
 - During polarization, $S_1 -$ to S_2 , 0.03 ma.
 - After polarization off. Another inactive caffeinized brain.
 - During polarization across pallium, $P -$ to V , 0.02 ma.
 - 5 sec. after polarization off. (No other waves followed those shown.)
- B. The influence of cephalo-caudal polarization on caffeine waves. Simultaneous PV (top line of each pair) and S_1S_2 (bottom line) recordings. a and d at speed shown in a; others at speed shown in b. (Note that the PV wave starts definitely after the S_1S_2 wave in a, b, and f, before it in c and d.)
- Caffeine wave. Arrow points to $S_1 +$ to S_2 notch.
 - Same, faster recording.
 - During surface polarization, $S_1 +$ to S_2 , 0.03 ma.
 - 5 sec. after polarization off.
 - 15 sec. after d.
 - 15 sec. after e.

e and f) the original relations are restored. The sign of the surface wave during and immediately after polarization is not simply reversed from that earlier or later; perhaps because of the extra complication of an altered, possibly inverted, PV potential due to the polarization and of changed diphasic interference.

Shifts of the locus of origin of the traveling waves, aside from any effects of polarization, also make uncertain the determination of changes in their velocity. Actually the situation in the whole isolated brain may be quite complex, with the main wave traveling from the olfactory bulb to the occipital pole and with secondary and satellite waves "firing back" from the latter. This is indicated by the times of appearance of the various waves at two sets of paired surface electrodes, and by the fact that cutting away the occipital pole regularly changes a polyphasic main wave in the anterior portion to the typical simple diphasic one and eliminates entirely the following satellite waves.

DISCUSSION

The new observations here reported were mainly suggested by the hypothesis outlined, and they support it as a whole. We do not claim, however, that all facts are accounted for nor that the present crude picture will prove adequate. The spontaneous reversal of the PV potential, without wave inversion, is especially disturbing. *On the other hand, the existence of a somatic potential, and the consequent electric fields playing upon masses of neurones, would offer an explanation of many other puzzling neural phenomena.*

It has been pointed out elsewhere (Gerard, 1941) how the mass functioning of the cortex in learning (Lashley, 1929) and of the neuraxis in development (Coghill, 1940) or regrowth (Weiss, 1936), the establishment of new functional neural connections between brain centers in conditioning, the existence of synchronized action of many neurones in the retina (Adrian and Matthews, 1928) or brain (*e.g.*, Jasper, 1937) or of other cells not in physical contact (*Nitella*, Hill, 1939), the decrease of neural metabolism associated with central inhibition (Dann and Gardner, 1930), the summation of excitatory and inhibitory impulses reaching separate neurone processes (Gerard, 1932), etc., are accounted for in terms of intercellular potentials and currents more satisfactorily than in terms of nerve impulses conducted along anatomically set pathways.

Further, much evidence is accumulating to show that action currents in nerve fibers can influence the threshold of the same fiber beyond a block (Hodgkin, 1937; Lorente de N6, 1939) or of a separate but nearby fiber (Katz and Schmitt, 1940; Blair and Erlanger, 1940), or can even excite responses under these conditions (Erlanger and Blair, 1940; Tasaki, 1939; Jasper and Monnier, 1938; Feng and Li, 1940).

There are good physical reasons for expecting more marked effects in the case of cell bodies. These are regularly free of myelin and so more accessible to intercellular currents; and their considerably greater size would expose them to a greater voltage drop in a given potential field than would be the case for a fiber. Also, as a current generator, the more separated poles of a somatic potential, as compared to that across a nerve membrane, would cause a greater current flow at a distance from the battery in the volume conductor. Offner and Winternitz (unpublished) have found, for the inverse case of a pick-up electrode, that the fraction of a fixed potential picked up in

a volume conductor decreases as the square of the distance from the source with a monopolar electrode, as the cube with a concentric one. And the finding that the caffeine wave crosses a complete anatomical section permits only an electrical explanation of some sort.

The measured PV potential is not proof of the existence of the postulated somatic potential. The former might be due to potential gradients in other structures than neurone somas (as ependyma, glia cells, nerve fibers, etc.), or to potentials between cell layers rather than within cell units. Experiments in progress with microelectrodes should make the interpretation more certain;* but if the measured potential is in fact that along the neurones' axes, many of the observed phenomena receive an easy explanation, and we shall assume that such is the case. Many details of the caffeine waves, however, are not simply accounted for on these lines and it is not anticipated that the present interpretation will remain adequate without later accounting for further complexities which are at present neglected.

If the main surface positive wave represents a discharge of the somatic potential, for example, what is the basis for the following negative wave, or the occasional preceding negative notch (Fig. 3c, 4d, 5f and g), or the secondary positive wave (Fig. 4g and h, see arrows), or the absence of the main wave with the presence of only the negative variation which normally follows (Fig. 3e and h, 4g and h). The regular negative wave behaves in general as if it were an after-potential, yet what seems to be this wave can be present with the preceding positive wave appearing or disappearing (e.g. Fig. 4h to j). Another type of negative wave seems unrelated to any positive potential and may appear as an alternate to it (Fig. 3c, d, and possibly e). This wave does not travel (the other nearly always does), but is encountered simultaneously—within 10 msec.—at electrodes widely spaced on the pallium.

Whether these latter potentials are due to other units than those responsible for the ordinary moving waves, or whether the same units can manifest potentials of opposite sign (as claimed for the retina, Granit and Helme, 1939, and for sympathetic ganglia, Eccles, 1936, see also 1939), we cannot say. On the first interpretation, deep units becoming active, and so negative as usually pictured, would probably account for the surface-positive wave, superficial units for the negative one. There is evidence that the positive and negative waves obtained from the mammalian cortex by electrical (Adrian, 1936) or strychnine (Dusser de Barenne and McCulloch, 1938) stimulation do depend on such separate cell layers. The results on frogs, however, do not fall into such a picture. For example, when a brain is placed in caffeine it would be anticipated that the superficial layers would be earliest affected and the surface-negative wave appear first—as is the case with strychnine in the cat—but this does not happen (e.g. Fig. 2A). Also, polarizing P positive to V should, if anything, differentially depress the superficial layer in the anodal region, yet it increases the negative wave. Nor is there

* In experiments to date, carried out by Alene Silver, no sudden voltage changes have been encountered at the depth of the layers of cell bodies.

any explanation, in terms of layers, of the reversal of sign of the caffeine waves with that of the PV potential.

Some further evidence bears upon two related problems; of the initial surface negative notch (which can occur even when P is normally negative to V—Fig. 3a, c, d, and g), and of the occasional presence of caffeine waves of normal sign with an inverted PV potential, or vice versa (Fig. 3c, d see arrow, and g). When the PV potential is followed continuously, it has been observed that a relatively steady potential, P positive to V, may swing rapidly to the normal direction (P negative), hold the new value for some seconds, and then return to the original conditions. Such shifts last about as long as a single train of caffeine waves and are repeated at the intervals at which these trains occur in the same brains. Whatever such PV shifts may mean, they seem rapid enough to give the initial negative notch on the "leader" wave (to which this notch is limited), and they could account for cases of apparent conflict between the signs of the PV potential and the traveling waves.

A final point may be made on the influence of polarization upon the caffeine waves and the PV potential. During the flow of applied current both alter together as expected, but when the current is stopped the steady potential falls off progressively towards the initial state while the waves often show a prompt "rebound" in the opposite direction (see also Gerard and Libet, 1940). Clearly other factors than the magnitude of the PV potential are involved, a change in cell irritability—as in polarized nerve—comes to mind.

Despite such additional problems, however, it is established that steady potentials do exist among brain cells and significantly influence their behavior. When these potential gradients alter, spontaneously or under the influence of applied currents, there occur changes in the oscillating potential waves—both traveling and stationary—which can be rationalized by the interpretations here developed. These changes include: their appearance or disappearance; a shift in their site of origin; their magnitude and sign; etc.

The probable importance of intercellular currents in synchronizing the action of many neurones has been emphasized earlier (Gerard, Marshall and Saul, 1936; Gerard, 1937; Libet and Gerard, 1939), and their role in producing traveling waves is here elaborated. The phenomena of electronarcosis (Silver, 1939) and electro-"epilepsy" are probably related. The focal origin of true epileptic seizures and their progressive spread do strongly resemble the phenomena in the caffeinized frog brain. The failure of waves, induced by strychnine (Dusser de Barenne and McCulloch, 1937) or accompanying epilepsy (Erickson, 1940), to travel across a cut in the mammalian cortex may indicate different mechanisms in these cases or may result from the relatively poor apposition of the cut surfaces achieved in a large brain *in situ*, subject to pulsations and bleeding at the cut.

What structural and metabolic machinery within a cell keeps charging the somatic potential battery despite the intercellular current leakage; and

what the relation is between the somatic potential and that recorded between different surface points of the neuraxis (see also Burr and Harman, 1939, who find the rat hemisphere as much as 16 mV positive to the sciatic; and Burge, Koons, and Burge, 1940) are questions for the future. Similarly, for the mechanism by which caffeine and like agents evoke traveling waves; although the evidence of decreased polarizability and steady potentials in the caffeinized brain suggest an action on membrane impedance (see Spiegel and Spiegel, 1939).

Finally, the somatic potential may supply the needed factor of integration for activity of a single neurone. The problem of how impulses reaching different dendrites of a cell can sum their influences, and the more difficult one of the summation of excitation and inhibition, have concerned many workers. If all synaptic potentials contribute to a change in the overall somatic potential, to alter it in one direction for excitation and in the other for inhibition, these difficulties are overcome (Gerard, 1941). There is indeed evidence: that nerve cells can manifest large spontaneous potentials over cell dimensions, and evoked potentials can be of either sign, depending upon where incoming impulses reach the cells (Renshaw *et al.*, 1940); that a change in potential of the axonic poles is related to the level of cell activity (Lorente de N6, 1939); and that an anatomically polar distribution of particular synapses exists for the Mauthner cell (Bodian, 1937).

SUMMARY

The continued travel, after neural connections are blocked or severed, of potential waves along the hemisphere of the isolated frog brain, depends upon the existence of intercellular currents. These are assumed to result from the existence, and discharge, of a potential, the somatic potential, from axonic to dendritic pole of the similarly oriented neurones. Such a polarized sheet of cells should behave formally like a polarized nerve membrane; and a number of predictions from the hypothesis have been tested and confirmed.

Steady potentials, relatively constant in time, have been observed between various surface points and, especially, between the uninjured pial and ventricular surfaces of the hemisphere wall. The PV potential is normally 2 to 3 mV, with P negative to V, but may slowly change magnitude and even direction, especially under the influence of caffeine.

The traveling wave induced by caffeine is simpler and of greater amplitude as recorded from PV electrodes than from surface ones. Its characteristics are described; essentially, a large surface positive wave is followed by a feeble and longer surface negative phase.

Polarization through the thickness of the pallium, by as little as 0.01 mA for a few seconds, changes the PV potential in either desired direction. The new potential returns to the initial value during 10 to 30 min. after the applied current is ended, but shows an asymmetry with the direction of polarization. The caffeinized brain is less "polarizable."

As the PV potential is altered, spontaneously or by an applied current,

the caffeine wave alters with it; and the two commonly reverse sign together. Some but not all exceptions are explained in the text.

"Depolarizing" the PV potential at some position on the hemisphere can initiate caffeine waves in a previously quiescent brain or can shift the source of waves already present so that they originate at this locus.

These findings support the hypothesis advanced. Some limitations and extensions of an interpretation of neurone interaction in terms of electric currents are considered.

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FATIGUE AND REFRACTORINESS IN NERVE

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IN A PRELIMINARY COMMUNICATION (Brücke, Forbes, and Early, 1940) we reported that prolonged repetitive stimulation of medullated nerve delays the recovery of responsiveness (size of maximal response) in the relative refractory phase, and to a much greater extent delays recovery of excitability. The present report amplifies our observations on these points and brings out some further information on fatigue and refractoriness.

METHOD

Single shocks used for conditioning and testing stimuli were delivered by Harvard coils to excised frog sciatic nerve as described in our previous paper (1941). Measured control of the strength of the testing stimuli was obtained by varying the resistance in the primary circuits. In experiments in which

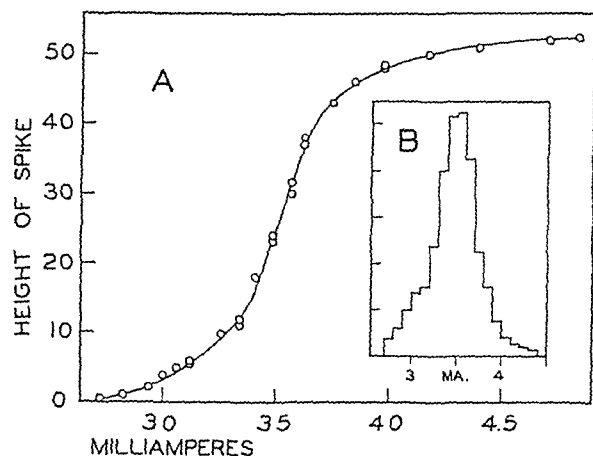


FIG. 1. A, height-intensity curve (height of spike against strength of stimulus) of resting sciatic nerve. B, distribution of α fibers according to thresholds, derived from A. (Frog, 11/6/40. Temp. 23°C.)

were led through a Grass condenser-coupled amplifier and registered on a cathode ray oscillograph adjusted for a single sweep which was controlled by the Lucas pendulum to start simultaneously with the conditioning shock or somewhat earlier.

In a many-fibered nerve excitability cannot be measured directly, as is possible, for instance, in a single fiber or in the heart. The heights of the responses cannot be used of themselves as an indication of excitability because there is no proportionality between height of response and strength of stimulus. In this paper calculation was generally made by the method of Graham and Lorente de N6 (1938) from the height of submaximal spikes according to their position on a height-intensity curve which was taken immediately before the fatiguing series or as soon as the nerve had completely recovered. In this way "it was possible to determine the strength of shock to which the constant testing stimulus had been made equivalent by the conditioning process" (*loc. cit.*, p. 327). The ratio of the actual strength of the test shock to the strength to which it became equivalent after fatigue provides a quantitative percentile measure of the decrease of excitability. If, for instance, the actual strength of the stimulus used was 5 mA. and the strength to which it had be-

prolonged activity of the nerve was required, a photo-electric cell provided the fatiguing stimuli, generally at a frequency of 240 per sec. In later experiments the fatiguing shocks were delivered through a one-to-one transformer to assure the avoidance of direct-current components. The fatiguing stimulation was interrupted by the opening of the first key of a Lucas pendulum, the opening of the second or third key inducing the testing shock. Usually the interval between the cessation of the fatiguing stimuli and the test was 80 msec. Further tests could not be made at intervals shorter than 2.5 sec. Testing and fatiguing shocks were always sent through the nerve in the descending direction through two separate pairs of Ag electrodes (see inset Fig. 4). The action potentials

come equivalent after fatigue was only 4 mA., we may say that excitability had decreased to 80 per cent. In this case an increase of the actual stimulus by 25 per cent to 6.25 mA. would compensate for the decrease of excitability, that is to say, it would bring back the least excitable fibers that had dropped out after excitability had decreased.

With regard to this indirect method of calculating excitability it might be of interest to consider that the smoothness of the height-intensity curve of the α fibers in a nerve is not self-evident. The distribution of fibers in the saphenous nerve according to diameter is so irregular (cf. Gasser and Grundfest, 1939) that thresholds of the fibers in a mixed nerve—generally linked to size—might be expected to be distributed irregularly also. This is not the case. Figure 1A shows a height-intensity curve taken from a resting nerve. In B the differences in height of responses to stimuli of increasing strength are plotted against stimulus strength. This curve (B) represents the distribution of α fibers according to their thresholds. We must bear in mind, however, that it is somewhat distorted because the height of the spike does not change in strict proportion to the increase in number of fibers responding. Except for a slight asymmetry this curve corresponds sufficiently well to a normal curve of distribution. In a few experiments the above method was compared with the classical scheme of varying the strength of the stimulus until the response was the same as that obtained before excitability had changed. Although both methods gave similar results, finer changes in excitability were detected by the classical procedure (direct method).

RESULTS

To avoid confusion between the lasting effects of prolonged stimulation and the transient refractory phase following a single response, we shall speak of the former as "fatigue" and shall use the word "conditioned" to denote the effect of a *single* preceding impulse. The word "resting," when used, signifies unconditioned, without reference to recent prolonged stimulation.

Recovery of excitability after prolonged activity. The combined effects of prolonged activity and refractoriness may be studied by recording recovery of the spike potential (i) at a constant interval after a conditioning shock at different times after the end of the fatiguing stimulation or (ii) at a fixed time after the end of the tetanization with a variable interval between the conditioning and the testing stimuli. The first of these methods was almost invariably employed; the curves in Fig. 11, however, were obtained in the second way.

Figure 2 is the photographic record of the recovery of a conditioned submaximal response after fatiguing stimulation, the interval between the conditioning and the testing stimuli being held constant. When in successive experiments an unconditioned submaximal spike potential and a conditioned submaximal spike potential were reduced by fatigue to the same height, they were both found to follow similar courses of recovery; but a thorough investigation of this point was not made.

In another group of experiments we determined the strength of stimulus required to evoke a submaximal unconditioned response of a fixed height. The strength of stimulus was then increased until a conditioned response of the same height was obtained. These two stimuli, the stronger for the conditioned response, the weaker for the unconditioned, were used to test the reduction of excitability after fatiguing tetanizations of a given length. It was found that excitability was reduced much more in the resting (unconditioned) than in the refractory (conditioned) nerve. Reduction of excitability in the

latter case depended upon the interval between the testing and the conditioning shocks; the longer this interval (that is, the weaker the testing shock required), the greater was the depression due to the fatiguing tetanization. An experiment of this kind is represented in Fig. 3. Excitability was reduced by fatigue to about 96 per cent of normal when the nerve was tested with strong shocks 2 and 3.2 msec. after the conditioning shocks, and to about 88 per cent when tested with a weaker shock later during the refractory state.

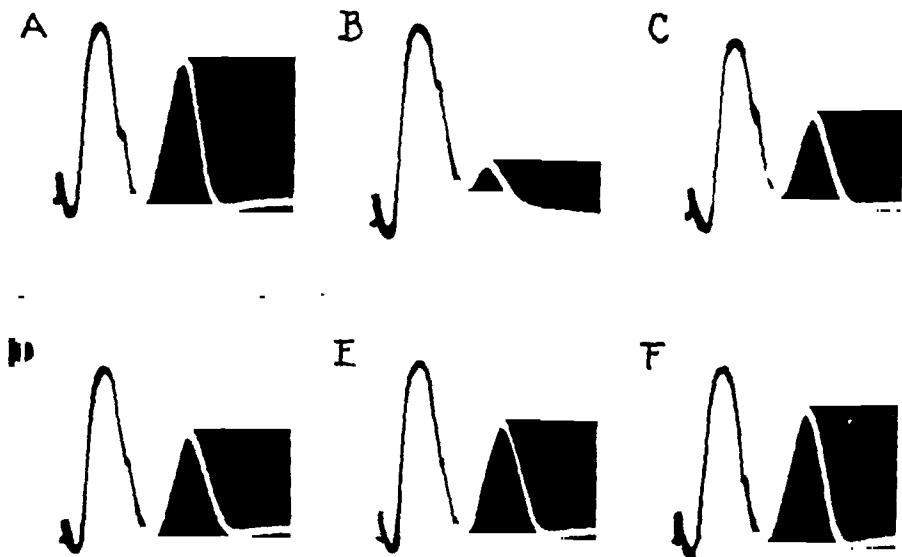


FIG. 2. Recovery of a conditioned spike potential with testing shock submaximal. Interval between conditioning and testing shocks 1.5 msec. A, response in unfatigued nerve, pictures B to F taken 40 msec., 6, 26, 80 and 140 sec. after the end of a 15 sec. tetanization (120 per sec.). The shifting of the artefact of the second induction shock along the descending part of the first spike potential indicates the increased duration of this spike after tetanization. (Frog sciatic nerve.)

When the unconditioned response to a *single* testing shock was observed, however, excitability was found to have dropped to from 80 to 83 per cent.

It became evident that there was need of investigation of the effects of prolonged activity on excitability in nerve in the resting state. Hence, experiments were performed which gave the following results. Rate of recovery of excitability depends directly on the duration of the fatiguing stimulation. This is shown in Fig. 4, where recovery of excitability to 95 per cent takes place at 16, 20 and about 100 sec. after tetanizations of 3, 5 and 15 sec. respectively. These results recall the fact that the "second" positive after-potential increases in duration as well as in size as the length and the frequency of the tetanus are increased (Gasser, 1939). According to Gasser and Grundfest (1936) the positive after-potential in a warm-blooded nerve was

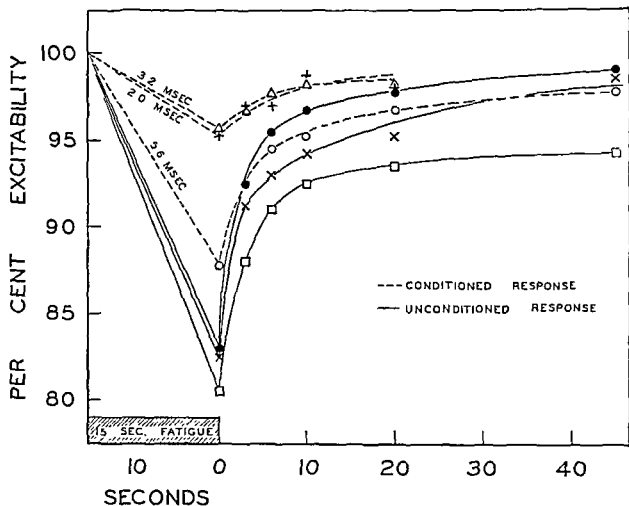


FIG. 3. Recovery of excitability after 15 sec. fatiguing stimulation. In each experiment the testing shock before fatigue provoked a submaximal response of identical height on the screen.

Dots, squares and x's, using single testing shocks (10:16, 10:33 and 11:02). Intensity in primary circuit: 9.6, 10 and 10 mA.

Crosses, triangles and circles, using conditioned stimuli after intervals of 2.0, 3.2 and 5.6 msec. Intensity in primary circuit: 67, 30 and 13.5 mA.

(Frog sciatic nerve, 10/7/40. Temp. 23.8°C.)

visible for 1 to 2 min. after a 10-sec. tetanization and for more than 4 min. after a 30-min. tetanization.

Experiments such as the one represented by the curves in Fig. 5 show that fatigability does not vary in different α fibers of a given nerve. These curves are height-intensity curves of one nerve taken (i) in the unfatigued state, (ii) after 5 sec. and (iii) after 10 sec. fatigue. The relative excitability in the fatigued nerve was calculated for five different heights of response by comparing the strength of stimulus (mA.) necessary to obtain each of these responses with the stimulus required for an equal response in the resting nerve. At all the five points considered, excitability was reduced by the two fatiguing periods in approximately the same relation (100:84:80). Since the ratio of the strengths of stimuli necessary before and after fatigue to obtain a certain height of response is a measure of the loss of excitability, and this loss is found to be the same at all points along the height-intensity curve after a fatigue of a given duration, the effect of fatigue is concluded to be the same for all α fibers of a given nerve.

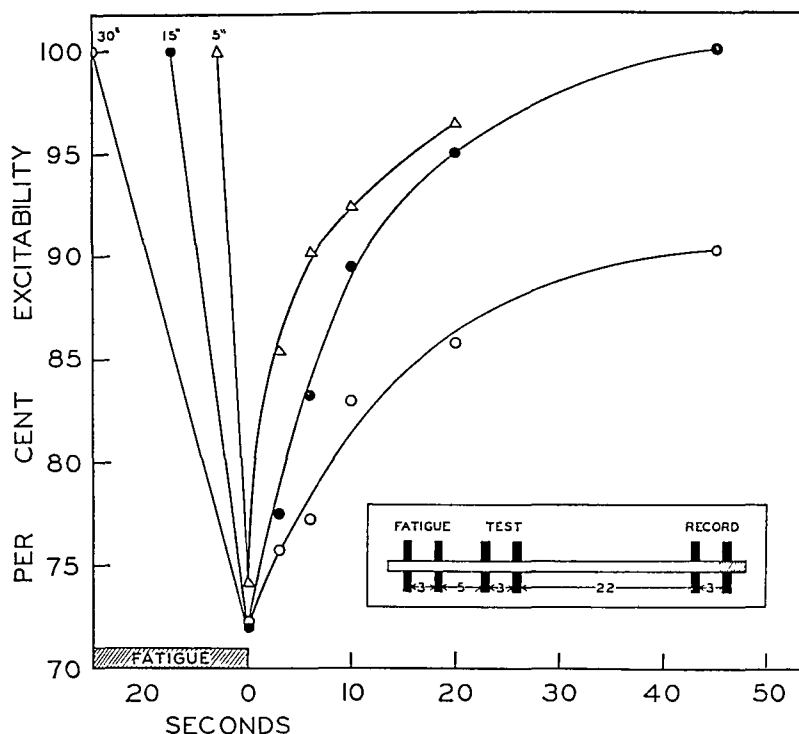


FIG. 4. Recovery of excitability after fatiguing tetani of 5, 15 and 30 sec. durations. (Frog sciatic nerve, 10/7/40. Temp. 23.8°C.)

Inset: Arrangement of nerve on electrodes; dimensions in mm

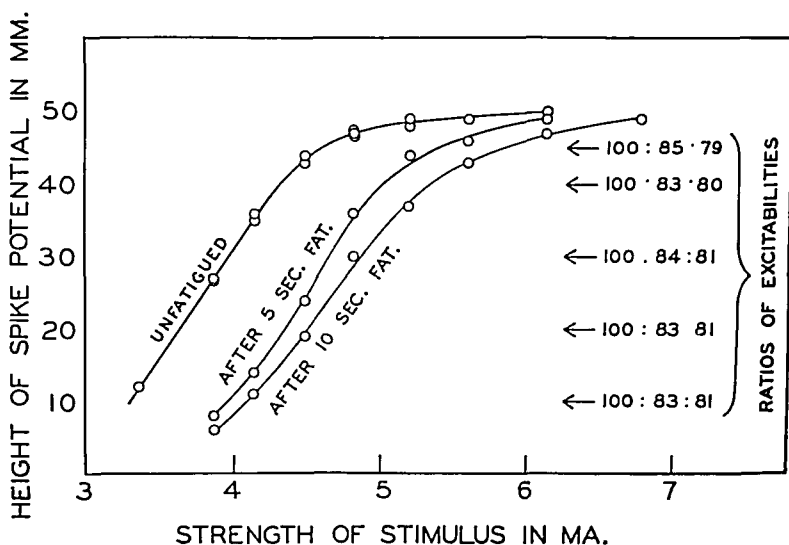


FIG. 5. Three height-intensity curves showing the same decrease in excitability in different groups of fibers after 5 and 10 sec. tetanizations. (Frog sciatic nerve, 2/24/30.)

After prolonged activity excitability often followed a steadily rising course (cf. Fig. 3 and 4) reminiscent of the delayed recovery seen by Graham and Lorente de N6 (1938, Fig. 5) in perfused nerves after very short periods of rapid tetanization. But in a number of nerves the rate of recovery was increased by the development of supernormality. Figure 6 shows 3 recovery curves of this type in a nerve which had been in the moist chamber for 2 hr. (dots), 2 hr. 49 min. (circles) and 3 hr., 11 min. (crosses). In these three experiments a tetanus of 15 sec. duration decreased excitability to from 81 to 84 per cent of its resting value. A marked supernormality with an early peak

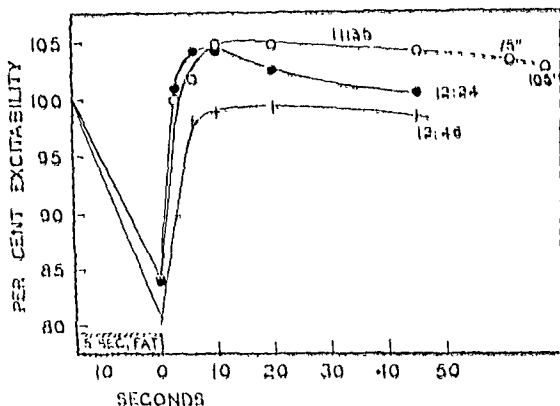


FIG. 6. Three curves of recovery of excitability, two showing supernormality. (Frog sciatic nerve, 10/1-10. Temp. 24.1°C.)

of excitability reaching not quite 105 per cent at about 8 and 15 sec. respectively after the end of the fatiguing stimulation is seen in the curves indicated by dots and by circles. In the last curve (crosses) no real supernormality is present.

In two experiments we had the opportunity of following the course of supernormality in recovery after tetani of varying durations. The curves of Fig. 7 illustrate one of these cases. They show also a subnormal phase following supernormality. As the duration of the fatiguing stimulation was increased, the appearance of the supernormal peak and of the subnormal depression was more delayed. This fact could only be indicated qualitatively in our experiments, since the intervals between successive tests could not be made shorter than 2.5 sec. After fatiguing stimulations of 2, 5, 15 (and 30) sec. durations the peaks of supernormality occurred at about 3, 5 and 7 sec.

after the end of fatigue; the subnormal depressions reached the minimum at about 6, 10 and 25 sec.

Gasser and Grundfest (1936) described the polyphasic return of excitability after tetanic stimulation in mammalian A fibers. From their Fig. 15 it appears that they observed the peak of supernormality to occur somewhat earlier than we found it in frog nerves as shown in Fig. 7. Their statement

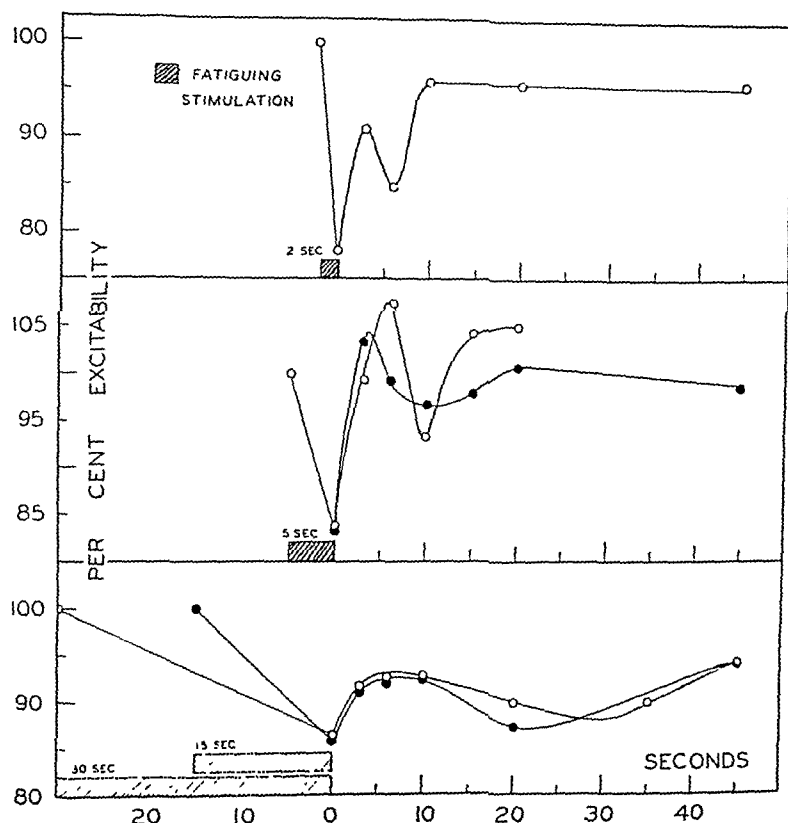


FIG. 7. Recovery of excitability in the same frog sciatic nerve after 2, 5 (dots and circles), 15 and 30 sec. tetanization (240 per sec.) showing increasing delay in the appearance of supernormality and subnormality. Nerve in moist chamber at 2:15; curves in above order taken at 2:50, 2:55, 3:00, 3:08, and 3:15 (10/10/40. Temp. 24°C.)

that the course of recovery partly depends upon the degree of previous activity is well illustrated by our results.

Recovery of excitability in different nerve fibers after activity—in spite of individual differences—on the whole shows the same trend. This is true for recovery after a single response as well as after a longer repetitive action. As a rule the following periods may be distinguished: (i) absolute refractoriness, (ii) start of recovery, (iii) supernormality, (iv) subnormality, (v) return to the normal (with occasionally a few further oscillatory changes occurring before complete recovery). The main difference between different nerves and

between nerves under different conditions lies in the spacing of these periods in time. For instance, let us follow in different nerves the time elapsing between the (last) conditioning response and the summit of the consequent supernormal period. It lasts about 7 msec. in mammalian A fibers (Gasser and Grundfest, 1936), 5 to 10 msec. in frog A fibers (Graham, 1934; Hayasi and Rittler, 1934), about 25 msec. in the sympathetic fibers for the frog

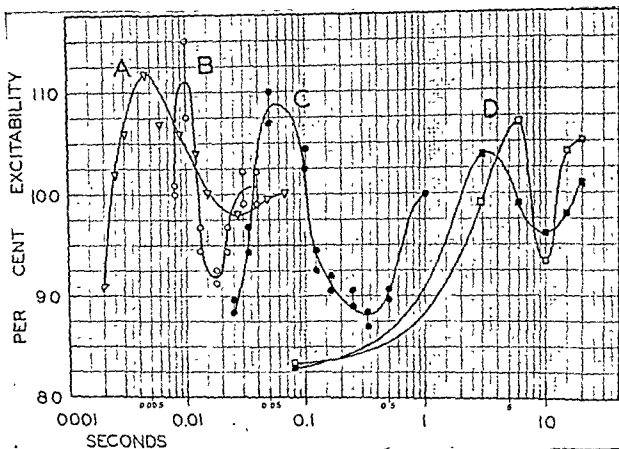


FIG. 8. Recovery of excitability with supernormal and subnormal phases in different nerves.

A. Saphenous nerve of the cat *in situ* (taken from Gasser and Grundfest, 1936, Fig. 18, top curve).

B. Sciatic nerve of the frog.

C. Preganglionic sympathetic pupillomotor fibers of the cat. B and C were drawn from chronaxie measurements by Hayasi and Rittler (1934, Fig. 2 and 4); their ordinates in this presentation therefore are arbitrary.

D. Sciatic nerve of the frog after 5 sec. stimulation (240 per sec.); both experiments on the same nerve (taken from Fig. 6 of this paper).

heart (Hayasi and E. Th. Brücke, 1934*), about 50 msec. in the preganglionic sympathetic fibers for the pupil in the cat (Hayasi and Rittler, 1934; F. Brücke, 1934), and 3 to 15 sec. in fatigued frog nerves (present experiments). Figure 8 includes in a single survey curves from these various papers. Some were taken from experiments in which chronaxie of different nerves was studied, using stimuli of different frequency. Such measurements

* It may be mentioned here that at the time these experiments were performed in my laboratory the phase of increasing duration of chronaxie was mistakenly connected with supernormality, while it is now evident that supernormality corresponds to the period with the *shortest* chronaxie.—E.B.

of chronaxie proved a very useful method of mapping the course of excitability.

Recovery of excitability after two conditioning stimuli. Whether or not late subnormality is simply a prolongation of the relative refractory period was discussed by several authors (Graham, 1935; Erlanger and Gasser, 1937; Brücke, Early and Forbes, 1941). It was pointed out that quick recovery of responsiveness during the refractory state favors an affirmative answer (Brücke, Early and Forbes, 1941, p. 90). But there still remained one objection to this assumption: Experiments with repeated stimulation (Kato, 1926) seemed to show that a nerve stimulated during its relative refractory period "recovers from the second response along exactly the same curve as if there had been no preceding response." In other words, refractoriness would not sum, whereas subnormality from successive stimulations does (Graham, 1935). As far as we see, Kato, working with nerve-muscle preparations, did not map the course of recovery of excitability in the "first" and "second" refractory state, but with a stimulus 10 times maximal measured the length of the "second least interval" at which the third stimulus would give a "summed" response. This may have revealed not the threshold of the nerve but the stage of recovery at which the subnormal nerve impulse could excite the muscle.

It therefore appeared necessary to make a comparison of recovery of excitability after a single conditioning impulse with the recovery in the so-called "second" refractory state (that is, during refractoriness following a response which falls itself in the relative refractory state of an earlier maximal impulse). The usual recovery curve of excitability was taken in isolated frog nerve, single submaximal testing shocks being applied at varying intervals after single conditioning shocks. Recovery in the "second" refractory state was observed in experiments in which *pairs* of maximal conditioning shocks separated by a fixed interval were followed by single submaximal testing shocks after the same intervals as in the first procedure. Although the "first" interval, that between the two conditioning shocks, was held constant for a given experiment, in different experiments it was varied between 1.3 and 5.5 msec. The variation of the "second" interval, between the second conditioning shock and the testing shock, furnished the points on the recovery curve. Both conditioning shocks were 5 to 10 times maximal; the strength of the submaximal shock was adjusted to give a response about $\frac{3}{4}$ of maximal in resting nerve. Excitability at different points along the curves was measured directly by increasing the strength of the testing stimulus at each interval until it evoked a response equal to the chosen magnitude of submaximal response in resting nerve. In these experiments a marked difference between recovery of excitability in the "first" and in the "second" refractory state was always found. This may be seen immediately from the different heights of response obtained at the same testing intervals in both refractory states, those in the "second" being smaller than the ones in the "first."

In Fig. 9 photographs of four pairs of responses are reproduced, the upper

row (A to D) showing recovery of height of the conditioned spike in the "first" refractory state, the lower row (A' to D') recovery during the "second" refractory state. The "first" interval in the pictures A' to D' was 1.7 msec., the "second" intervals used in the four pairs of tests were 2.7, 4.4, 5.7 and 9.3 msec. The submaximal testing shock was subthreshold for the β fibers. The response evoked by the testing shock alone (Fig. 9E) lacks the slight β elevation clearly seen in the descending part of the first conditioning spike. The second conditioning shock (A' to D') by summation excites a set of fibers with low conduction velocity eliciting a small potential following the second spike. In the descending part of the first spike the artifact of the second conditioning shock is seen. Using a "second" interval of only 2.7 msec.

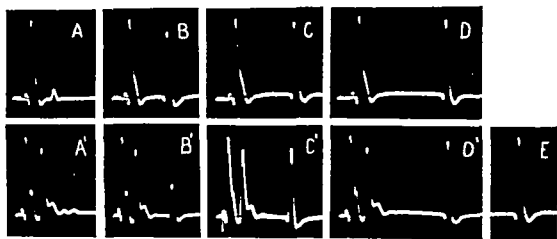


FIG. 9. Recovery of spike during "first" (A to D) and "second" (A' to D') refractory states. Intervals between conditioning and testing shocks in A to D: 2.7, 4.4, 5.7, and 9.3 msec. In A' to D' intervals between first and second conditioning shocks, 1.7 msec.; intervals between second conditioning and testing shocks as in A to D. E is the submaximal response to the testing stimulus in unconditioned nerve. (Frog sciatic nerve, 12/11/40.)

the small conditioned spike is very distinct in A; whether one of the two very small potentials in A' is due to the testing stimulus cannot be decided. In B and B' the difference between the responses to the test shocks is evident. In C and D measurements show that the doubly conditioned spikes are still 7 per cent and 3 per cent smaller than the singly conditioned ones. In this experiment 97 per cent excitability was reached in the "first" refractory state at about 3 msec. after the testing shock and in the "second" refractory state after about 9 msec.

As the "first" interval was increased, recovery in the "second" refractory state was found to become faster (Fig. 10A and B). Only 8 frogs were used for these experiments, a number perhaps too small to allow a definite general statement as to the length of the "first" interval at which the two recovery curves become identical. With intervals between 1.3 and 2.7 msec. recovery always was found to be slowed down. In one frog at a "first" interval of 3.3 msec., recovery already had reached its normal duration. In another experiment on a nerve which showed very slow recovery, after a "first" interval of 5.5 msec. excitability recovered only slightly more quickly than after a 1.6

msec. interval and much more slowly than during the "first" refractory phase. The time required for full recovery of excitability in the "second" refractory state varied widely. In Fig. 10A full height of the response was attained after about 13 msec., while in B excitability evidently achieved its resting value much later. Excitability in these experiments was not tested at intervals longer than those necessary for full recovery; therefore it is not known whether or not supernormality was present under the given condi-

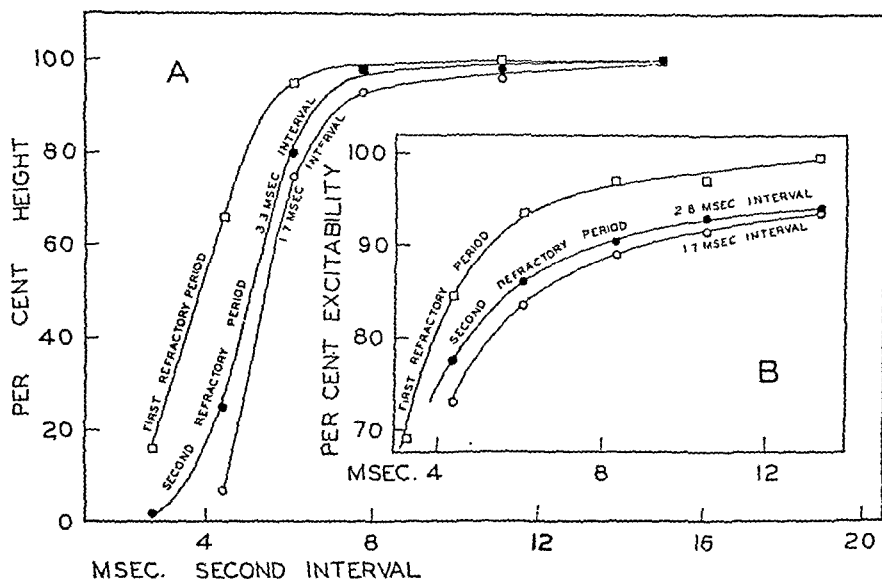


FIG. 10. Recovery of height (A) (Frog No. 2 sciatic nerve, 12/11/40) and of excitability (B) measured directly (Frog No. 3 sciatic nerve, 12/11/40) in the "first" and the "second" refractory states; testing stimuli submaximal. The intervals between the first and second conditioning shocks in the four experiments on the second refractory period are indicated on the curves.

The zero point in time for the curves of the first refractory state is at the moment of the conditioning shock; for the curves of the second refractory states it is at the moment of the second conditioning shocks. The curves have been drawn in this manner to facilitate comparison.

tions. The law according to which refractoriness increases when evoked by two, three or n conditioning stimuli has not yet been studied. However, it certainly depends upon the interval between the stimuli.

According to our experiments two conditioning stimuli altered recovery of excitability qualitatively in the same way as three or more stimuli did in the experiments of Graham and Lorente de N6 (1938), but in our excised frog nerves excitability was affected early in the refractory period, whereas in blood-perfused mammalian nerve this influence appeared only in the later part of recovery.

Recovery of responsiveness. The difference between the rates of recovery of responsiveness and of excitability observed in nerve by Graham and Lorente de N6 (1938) is still more accentuated in *fatigued* nerve. This may

be illustrated by the curves in Fig. 11. These curves show the height of the second response at different intervals after a maximal conditioning impulse. The recovery of responsiveness—shown in the two curves at the left—was tested with a *strong* stimulus, exciting *all* the fibers during the relative refractory state. In the unfatigued nerve the spike potential reached its full height after 2 msec. After the nerve was tetanized for 10 sec., recovery occurred somewhat more slowly, being complete after an interval of about 2.7 msec. The other two recovery curves give the height of the second response in

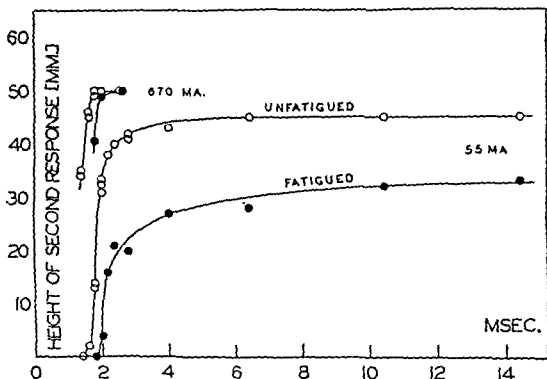


FIG. 11. Effect of fatigue on recovery of responsiveness (indicated by height of response to maximal testing shocks) and on recovery of excitability (indicated by height of submaximal spikes). Circles, recovery in unfatigued nerve; dots, after 15 sec. tetanization. (Frog sciatic nerve, 1/25/40. Temp. 24.6°C.) Strength of current in primary coil is indicated in the figure in milliamperes.

the unfatigued and the fatigued nerve tested with a just *submaximal* stimulus. Recovery of excitability in the unfatigued nerve is complete after 5 msec.; in the fatigued nerve the separation of recovery of excitability into two parts (Graham and Lorente de Nó, 1938) is marked and complete recovery occurs late.

When using unconditioned *supermaximal* testing shocks in studying recovery after prolonged activity in many nerves we met with higher responses immediately (80 msec.) after the end of the fatiguing stimulation than at 2 or 3 sec. later. This abnormally strong reaction to the first testing shock after the fatiguing stimulation was associated with a shifting of the baseline in the positive direction. In a few experiments testing shocks of 2 to 8 times maximal were used; the increase of height immediately after tetanization became more marked as the stimuli were strengthened. With weak testing stimuli, however, a small response or even none at all was seen, indicating that the abnormal height of response to the first supermaximal stimulus was not due

to increased excitability but to increased responsiveness. In Fig. 12 the second of the six spikes shows both the shifting of the baseline and the abnormal height of the spike; in the same figure changes of conduction velocity due to fatigue may be seen directly from the shape of the β wave. The relation of these findings to those reported by Forbes and Rice (1929) has not been thoroughly investigated. It is possible that their decreased size of response following the fatiguing stimulation was due to decreased excitability causing the stimuli to become slightly submaximal.

In some experiments in which testing stimuli of *medium* strength were used, the unconditioned response immediately after a fatiguing stimulation was smaller than before the tetanization, but it was not really reduced to the minimum, as the next testing stimulus, after 2 or 3 sec., provoked a still smaller response (cf. Fig. 13). It seems as if in these cases the dropping out

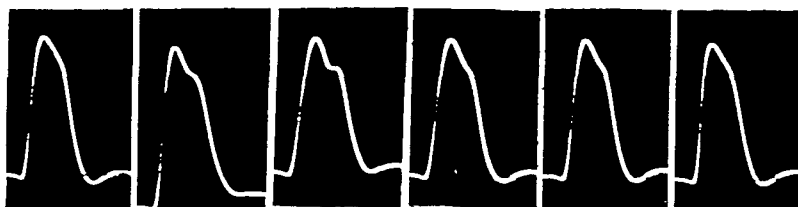


FIG. 12. Responses to supermaximal testing stimuli. From left to right: spike in resting nerve, spikes recorded at 80 msec., 2.5, 7.5, 10, and 12.5 sec. after 15 sec. fatiguing stimulation (240 per sec.). Testing stimulus 5 times maximal. (Frog sciatic nerve, 10/30/40.)

of some fibers because of decreased excitability was partly compensated for by an increase in responsiveness in the still responding fibers.

Since anodal polarization has long been known to increase the amplitude of the spike potential (cf. Bishop and Erlanger, 1926) we thought that perhaps a direct-current component in our fatiguing stimuli might be the cause of the phenomenon. Therefore in later experiments the fatiguing shocks delivered by the photocell were sent through a transformer before reaching the nerve. But with this arrangement, also, abnormally high responses were seen immediately after the end of the tetanization. There is also a possibility that the increasing spike magnitude corresponds with a period of positive after-potential after the fatiguing repetitive stimulation; no experiments were carried out to test this possibility. No abnormal height of response was observed during the refractory period set up in a fatigued nerve by a conditioning shock. The effect of the second (testing) shock in these experiments 80 msec. after the end of the fatiguing stimulation was always smaller than it was some seconds later.

Marked prolongation of refractoriness in fatigued nerve was first described by Field and Brücke (1926), who at that time thought that the *absolute* refractory state also was prolonged by a fatiguing stimulation. Woronzow (1935) later found an increase in duration of the absolute refractory

state (from $1\frac{1}{2}$ to 3 times its resting value) only after several hours' tetanization. He justifiably criticized Field and Brücke for having used stimuli that were below threshold for the most excitable fibers in the early relative refractory state. But Woronzow's statement that the *relative* refractory state also was not much prolonged after fatigue is probably due to the fact that he used *strong* testing stimuli, by which he studied the relatively quick recovery

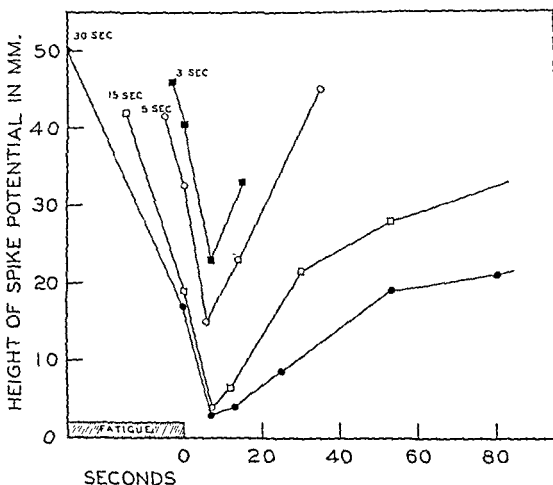


FIG. 13. Effect of fatiguing tetani of different durations on height of spike potential. Note that the first response after the end of fatiguing stimulation is higher than the next one following after about 5 sec. Rate of recovery parallels duration of tetanus. (Frog sciatic nerve, 1/15/40.)

of responsiveness rather than the recovery of excitability. It can no longer be doubted that recovery of excitability in nerve is greatly delayed by fatigue.

DISCUSSION

In the present paper it seemed advisable not to discuss experimental results in a separate section, but to include the discussion with the observed facts. Only a few points therefore will be mentioned separately here.

Nerve and heart. For the study of many physiological problems the heart may be used as a model for excitable tissue, but as far as its refractory state is concerned, it seems to behave in a slightly different way from nerve. The refractory state following an extrasystole is not prolonged, but shorter than the one after a normal systole (Trendelenburg, 1903; Umrath, 1925). Whether contractility of the heart muscle recovers during the relative re-

fractory state with the same speed as excitability, has not, as far as we know, been definitely determined. The data presented by Tigerstedt (1921) suggested that in the frog's ventricle excitability recovers more quickly than does the height of systolic contraction.

Refractory and subnormal periods. The period during which excitability returns to normal after a single response is called the "relative refractory period." Graham and Lorente de Nó (1938) from their experimental findings—confirmed in the present paper—suggested a division of the relative refractory state into two parts "during the first of which (1.5 to 2 msec.) height and velocity recover completely, and excitability recovers to an extent (roughly 80 per cent) that does not change significantly after rhythmic activity. The completion of the recovery of excitability during the second part of the cycle is greatly affected by rhythmic activity and other conditions." This second part of recovery—if followed by a supernormal phase—may end at the point where supernormality starts. On the other hand, the increase in refractoriness after a second conditioning stimulus and the fact that responsiveness increases no further during the second part of the refractory period are in favor of the assumption that *subnormality*, when observed after the supernormal period, is not to be considered a separate phenomenon; it is just a late continuation of recovery of excitability (cf. Brücke, Early and Forbes, 1941). Thus the refractory state may last for many seconds, even for minutes, yet the process of which it is the expression may be the same after a single nervous impulse as after prolonged activity (cf. Fig. 8). If the idea is correct that recovery of *excitability* during the refractory and the subnormal period is one and the same process, the difference in time between this recovery process and the quick restitution of responsiveness becomes still more marked. It might be useful to separate them by nomenclature. The term "relative refractory period" might be reserved for the first short period of recovery of responsiveness, whereas the expression "subnormal period" (Graham, 1935) already used for a relatively slow recovery of excitability, might be reserved for this process as a whole in whatever form it appears.

A difference between relative refractoriness after a single impulse and the slow recovery of excitability after prolonged activity may be seen in the fact that no positive after-potential has been found to accompany the relative refractory phase as it does subnormality after prolonged activity. This difference, however, is of hardly any importance because the positive potential in A fibers is so small that it could not be detected if it were present at the beginning of the relative refractory period, that is, during the steep descending part of the spike, and it could easily be masked by the negative potential at the end of the spike.

Elementary manifestation of fatigue. The depression in excitability due to prolonged stimulation is the main and often the only symptom of the condition that is generally called "fatigue" in nerve. Since this condition affects and prolongs the subnormal period which is assumed to be the same as the old "refractory" period, it is merely a matter of nomenclature whether we speak of a state of fatigue or of a prolongation of the refractory period. The delay of recovery during the "second" refractory state may be the first detectable sign of fatigue. But in going one step further we might also assume

that not only is *prolonged* refractoriness a symptom of fatigue in nerve but also that the short refractory state itself following a single impulse in resting nerve is the elementary manifestation of fatigue.

SUMMARY

Recovery of excitability of frog sciatic nerve was studied after one and two conditioning shocks, after prolonged tetanization and in experiments combining prolonged activity and refractoriness. Generally excitability was measured by the method of Graham and Lorente de Nó (1938), the theoretical foundation of which is discussed.

To compare the effect of similar fatiguing tetanizations on submaximal single responses and on conditioned responses the testing stimuli were adjusted before fatigue so that conditioned and unconditioned responses would involve the same number of fibers. It was found that excitability was reduced much more in the unconditioned (resting) than in the conditioned (refractory) nerve. In both cases the rate of recovery of excitability depends directly on the duration of the fatiguing stimulation.

Fatigability does not vary in different α fibers of a given nerve.

The appearance of supernormality and of subnormality was delayed by fatiguing stimulation, the degree of delay depending upon the degree of previous activity. Recovery curves of different nerves taken from various papers were plotted together on a semi-logarithmic scale. Thus in a single survey the different spacings in time of supernormality and subnormality are revealed. In a variety of preparations although the total duration of the recovery curve varies greatly, the sequences are similar (Fig. 8).

Recovery of excitability after a single conditioning impulse was compared with recovery during the so-called "second" refractory state (after two conditioning impulses). Recovery was always found to be slowed down in the latter case; as the interval between the two conditioning impulses was increased, recovery in the "second" refractory period was found to become faster. This delay of recovery during the "second" refractory period may be the first detectable sign of fatigue.

The fact that the separate refractory states after two impulses sum, just as subnormality does, and the fact that responsiveness increases no further during the second part of the refractory period (Graham and Lorente de Nó, 1938) are considered to favor the assumption that subnormality is a late continuation of the relative refractory state.

The difference between the rates of recovery of *responsiveness* and of excitability is accentuated in fatigued nerve.

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ACETYLCHOLINE ESTERASE CONCENTRATION DURING THE DEVELOPMENT OF THE HUMAN FETUS

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INTRODUCTION

PREVIOUS studies (Youngstrom, 1938) have shown a definite correlation between the choline esterase concentration and the functional development of the neuromuscular system in amphibian embryos. The present work is an extension of these studies to the human fetus which, because of its large size and slow development, is admirably suited to the purpose.

The specimens used were made available through the coöperation of the Department of Obstetrics and Gynecology, and were selected from cases of therapeutic abortion (hysterotomies and hysterectomies) with due regard to their behavior as established by personal observations and by comparison with the descriptions of human fetal behavior published by Hooker (1936, 1939). The pharmacological assays performed upon their tissues have provided evidence of a correlation between the choline esterase content and the functional development of the nervous and muscular systems.

Nachmansohn (1940a) has studied the choline esterase content of several species of animals, giving data on rather scattered periods of their developmental life. In so far as his works have a bearing on the present study, they will be discussed later.

MATERIAL AND METHODS

As soon as a specimen was received from the operating room it was placed in warm saline for the exploration of its behavior and subsequent dissection. The parts selected for esterase determination were placed in individual Wassermann tubes in an icebox at 0°C.* until such time as the esterase determination was to be done.

The tissue was then ground in a Ten Broeck grinder with a definite amount of buffer of pH 7.61 and diluted with distilled water to a standard suspension. The amount of esterase was determined by the pharmacological method with the guinea pig ileum (Bernheim and Bernheim, 1936). Eight specimens were used which ranged in size as follows: 21, 33, 48, 51, 65, 100, 135, and 240 mm. live crown-rump length, respectively. Their menstrual age was calculated from their crown-rump length by applying the formula of Edith Boyd.†

RESULTS AND DISCUSSION

The results are presented in Fig. 1, each plotted point representing the acetylcholine esterase concentration of a particular part of one fetus. The

* As previously reported by Mann, Tennenbaum and Quastel (1939), the enzyme was found to be stable at this temperature.

† $A = \frac{83.259015}{2.820106 - \log L}$, where A is menstrual age in days and L is crown-rump length in millimeters (quoted from Hooker, 1936).

unit of enzyme activity is the number of mg. of acetylcholine iodide hydrolyzed by 100 mg. of tissue (dry weight) per hour at pH 7.61 and 38°C.

The youngest human fetus in this study, being approximately 56 days menstrual age, is almost ready to move (Hooker, 1936). The rapid expansion of behavior immediately following this period is paralleled by an equally rapid increase of the choline esterase concentration in the spinal cord, medulla and midbrain, in other words the region of the motor plate. This increase continues at least to 121 days by which time the fetus has almost all the specific reflexes of the adult except respiration (Hooker, 1936). The sequence by which the enzyme was found to increase in the different parts of the central nervous system (midbrain and medulla, spinal cord, diencephalon, basal ganglia and cerebral hemispheres) correlates very well with the order of morphological differentiation in the central nervous system (Coghill, 1929; Angulo, 1939). It is also in general agreement with the four phases of fetal development as outlined by Minkowski (1938) from his study of the response to plantar stimulation; however the present data suggest that functional development begins somewhat earlier in the several regions of the central nervous system than is indicated by him.*

In the case of the basal ganglia and of the cerebrum, there is but slight increase in the choline esterase concentration until after 121 days menstrual age, when a precipitous increase occurs. This suggests that differentiation of this region does not really get under way until after four months menstrual age, which agrees very well with the morphological differentiation in the human cerebral cortex as it has been described by von Economo and Koskinas (1925).

Because of its late development and small size, no determinations have been attempted with the cerebellum until 102 days menstrual age. By 189 days its choline esterase concentration has more than doubled and has almost reached the value for the adult cerebellar cortex.† The most rapid growth of the cerebellum comes after the seventh fetal month (Dunn, 1921).

The liver was the most concentrated source of choline esterase in the 56-day fetus. The highest values for this tissue were obtained when specimens between 56 and 75 days menstrual age were used. This is during the period when hemopoiesis is most active in the liver (Knoll, 1932), a fact which takes increased significance in view of the report that choline esterase is more concentrated in the cells than in the serum of the blood, and that the choline es-

* After the menstrual age of his fetuses has been calculated by applying Boyd's formula to their crown-rump length, his four phases would then be as follows: (i) Neuro-muscular transitional phase when the influence of the spinal cord and medulla is beginning to be exerted upon the muscles (68-74 days); (ii) The spino-bulbar phase (72-129 days); (iii) Tegmento-bulbo-spinal phase (135-243 days); and (iv) Pallido-rubro-cerebello-segmento-spinal phase (243 days till birth). The menstrual ages actually assigned to these periods by Minkowski are (i) about two months; (ii) 3 to 4 months; (iii) 4 to 6 months; (iv) 6 months to birth.

† The following results on adults were obtained with material secured from neuro-surgical cases through the cooperation of Dr. Barnes Woodhall: cerebral cortex 2.2, 0.9, 2.0, 2.1; internal capsule 1.18, 1.5; cerebellar cortex 28.

terase of the cells is qualitatively different from that of the serum (Alles and Hawes, 1940). It is apparently the red cells with which the enzyme is associated since washed pus cells (Ginsberg, Kohn and Necheles, 1937) and a practically pure mass of histiocytes from an area of a brain tumor* contained almost no esterase. The formation of bile salts in the fetal liver may be another factor in the eventual depression of this curve (Glick, Lewin and Antopol, 1940).

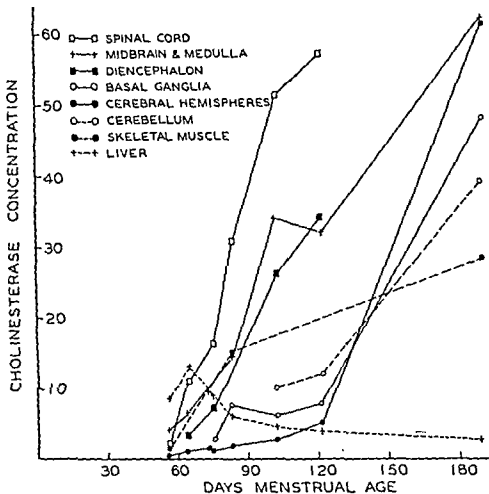


FIG. 1.

The most rapid concentration of choline esterase in the muscles of the back and shoulder comes when motility is developing in these parts (Hooker, 1936). This is also the period when the enzyme is being concentrated most rapidly in the spinal cord. Thus there is seen a close relationship between the period of rapid increase of choline esterase and the functional development of the neuromotor mechanism.

Since the endplates are not yet present (Dickson, 1940) when the skeletal muscles of sheep fetuses have the richest concentration of acetylcholine esterase, Nachmansohn (1940b) concluded that "the concentration is more closely related to the function than to the histological formation" (p. 401). He has not attempted precise correlation of the enzyme concentration with the developing behavior of the animals studied by him. Though spontaneous

* Unpublished observation.

movements begin as early as 34 days gestation in sheep fetuses (Barcroft and Barron, 1939) no data are given on the choline esterase content earlier than 60 days. In so far as correlations are possible, his results are in general agreement with the studies presented herewith on the human fetus.

Windle and Fitzgerald (1937) state that the spinal reflex arcs of the human are completed during the 8th week of intrauterine life; however, it appears difficult to demonstrate motility in the human fetus during and somewhat after, this period. Furthermore, there is no general agreement as to the correct interpretation of the movements observed. Some insight into this difficult problem is gained from a consideration of the neurohumoral mechanisms at this stage of development.

Kuo (1939) has shown that the most rapid increase in the concentration of acetylcholine in the chick embryo comes between the second and fifth day of incubation after which the concentration may be said to remain at the same general level if allowance is made for considerable fluctuation. Thus the acetylcholine concentration in the chick embryo has practically reached its maximum before there is any behavior. It would appear, therefore, that motility may be hampered in its development by a deficiency of the enzyme choline esterase rather than of the substrate, acetylcholine.

Assuming that acetylcholine is liberated by certain neurons as they begin functioning in the human fetus, the removal of the products of the action of choline esterase (acetic acid and choline) by the circulation may be at first deficient in some respect as has already been indicated in amphibia (Youngstrom, 1938). Furthermore, the proportion of electrolytes in the early stages may affect the choline esterase activity (Mendel, Mundell and Strelitz, 1939). Thus the accumulation of high concentrations of choline esterase in the fetus might be thought of as a compensatory mechanism. A clearer understanding of this mechanism should lead to a broader comprehension of developing motility and behavior.

SUMMARY

1. The acetylcholine esterase concentration in the several gross divisions of the central nervous system, in skeletal muscle and in liver has been determined on the human for the fetal period.

2. Correlation of these studies with the developing motility and behavior reveals a significant relationship. The period of most rapid concentration of choline esterase in the several gross divisions of the central nervous system seems to parallel their functional development.

3. The liver has its highest concentration of choline esterase in the early fetal stage.

4. A satisfactory explanation has not yet been found for the occurrence of a greater concentration of choline esterase in fetal than in adult tissues.

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LOCALIZATION OF CEREBRAL CENTER ACTIVATING HEAT-LOSS MECHANISMS IN MONKEYS*

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THERE is considerable evidence to indicate that an elevated intracranial temperature constitutes an effective stimulus for the activation of heat-loss mechanisms. The supposition is that the increase in the degree of cerebral heat is due to a rising temperature of the blood, a premise which has received substantial experimental indorsement. Warming of the carotid blood has been shown to cause sweating, peripheral vasodilatation and polypnea in various animals (Kahn, 1904; Moorhouse, 1911; Hammouda, 1933). This testimony has been upheld by the results obtained on direct heating of the brain, whether by circulating hot water through a closed tube inserted into the region under investigation or by open irrigation with warm fluids. By such methods, several workers have produced a fall in body temperature, sometimes accompanied by vasodilatation (Barbour, 1912; Hashimoto, 1915; Prince and Hahn, 1918).

In these early researches the corpus striatum was thought implicated and adjudged to be the "heat center." However, Sachs and Green (1917) could not confirm this hypothesis, while Moore (1918), though able to induce an antipyretic effect by warming the forebrain, determined that the corpus striatum was not specifically responsible. In the light of subsequent studies, it is probable that the positive results elicited from the striate body were due to the spread, to other areas, of the prolonged and intense heat applied in these experiments.

Attention was directed to the possible rôle of the hypothalamus and adjacent structures by Hasama (1929), who provoked profuse sweating and a fall in body temperature by warming the base of the brain in this vicinity in the cat. Supportive though inconclusive evidence was provided by Hammouda's (1933) demonstration that injection of warm saline into the third ventricle caused panting in the dog. In an attempt to delineate the heat-sensitive region with greater precision, Magoun, Harrison, Brobeck and Ranson (1938) resorted to the more refined technique of heating the brain locally by a diathermy current passed between electrodes placed at desired loci with the Horsley-Clarke instrument. They outlined, in the cat, a discrete field, heat-stimulation of which caused respiratory rate increase, panting and sweating. This center was situated between the anterior commissure and the optic chiasma, and there was a zone of lesser response extending caudally through the diencephalon to the midbrain. In 1940, Hemingway,

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Rasmussen, Wikoff and Rasmussen essentially repeated the experiment of Hasama (1929), heating the base of the brain in the unanesthetized dog with a high-frequency current conducted by a plate electrode implanted over the optic chiasma at a previous operation. They were thereby able to inhibit shivering and induce vasodilatation, but not to obtain panting.

The present study on the monkey was undertaken in order to examine the problem in an animal of a higher order so as to throw more light on the question of the central control of body temperature in man.

METHODS

Eighteen monkeys (*Macaca mulatta*) were employed in this investigation. Eleven of these were used only incidentally; they were normal animals of the colony which were subjected, for varying lengths of time, to environmental temperatures of 104° to 120°F., in order that the typical heat-loss behavior of the monkey might be determined.

Seven macaques were utilized for experimental heating of the brain. Anesthesia was produced by urethane, 0.8 gm. per kg. of body weight, injected intraperitoneally and occasionally supplemented with ether during the surgical preparation of the animal. No polypneic breathing, as is common in the cat under the influence of this narcotic, was observed in the monkey. Operative exposure was provided by a trephine opening made in the midline of the calvarium in the region of the frontoparietal suture and enlarged with rongeurs. The dura mater was widely incised and reflected. So that the midline might be explored, the superior sagittal sinus was in some instances compressed with a small metal clip to reduce its width and clear the field for introduction of the electrodes.

Two straight needle electrodes, 0.5 to 1.0 mm. in diameter, were mounted in the multiple holder of the Horsley-Clarke instrument, and the distance separating them in the transverse axis adjusted as desired between 2 and 8 mm. The electrodes were constructed of insulated, respectively, with enamel or to within 2 mm. of their pointed tips.

After the electrodes were inserted, they were fixed in position by means of a stereotaxic apparatus, after the technique of Ranson (1934). The electrodes were always inserted so as to be equidistant from the midline. The vertical and rostrocaudal steps in exploration were routinely spaced at 2 mm.

A tuned-plate, tuned-grid oscillator similar to that used by Magoun, Harrison, Brobeck and Ranson (1938) was employed. This delivers a current similar to that of the familiar diathermy machine, but of low power. In each experiment, an alternating voltage of high frequency (1 Mc.) and low intensity (14.2 V.) was applied to the electrodes for a 5-minute period. At conductance of the current between the tips of the electrodes and through the intervening tissue, the output voltage fell to an intensity in the neighborhood of 11.0 V. To determine how heating occurred during the passage of the current the temperature of the electrodes and of the brain substance in contact with the electrodes was measured in preliminary experiments on cats by using a thermocouple as one of the electrodes. It was found that equilibrium was established one minute after onset of current passage; a temperature gradient ceased to exist one half minute after the current was discontinued. Application of 14.2 V. to electrodes separated by 6 mm. raised the temperature of the tissue in contact with an electrode by 10.5°C. It was manifest that the temperature gradient with distance from the electrode surface was steep. Therefore, it can be assumed that a localized, approximately spherical heat field was centered about the tip of each electrode.

During each individual experiment, the amount of skin moisture, the respiratory rate and the rectal temperature were followed. Some of the work was carried out in a humidified room, in order that sweating might be more easily observed directly; the remainder under ordinary conditions of humidity. The room temperature was constant throughout, in the vicinity of 80°F. In certain animals sweating was detected by the iodine-starch indicator technique as described by List and Peet (1938). Between periods of heating the brain, the animal was allowed to rest for 5 min., or longer until sweating had ceased. The respiratory rate was counted at frequent intervals (1 min. or less). Rectal temperature was noted by thermometer or recorded continually by a Leeds and Northrup Micromax. Some attempts

were made to gauge alterations in the vasomotor state by measuring skin temperatures with locally applied thermocouples or with a Tycos Dermatherm.

The brains were serially sectioned in the plane of the punctures and stained by the Weil method. All points heated were located and plotted on a succession of projection tracings prepared from sections at one millimeter intervals on the formalin fixed brain. From this series there were selected the levels at 2 mm. intervals reproduced in Fig. 1, upon which were also plotted the reactive points from intervening levels. In most of the specimens there was little destruction (usually much less than 0.5 mm. in radius) around the electrode tracts.

RESULTS

Responses. The 11 normal animals exhibited stereotyped heat-loss behavior when exposed to high environmental temperatures and served to establish a norm with which the responses to local cerebral heating could be compared. As in the cat, heat-loss mechanisms were brought into play in the monkey only after a considerable rise in body temperature. In all animals the requisite rectal temperature level was slightly more than 103.0°F., at which point sweating began rather abruptly. Perspiration was marked on the palms (much more marked than that occasioned by excitement) and noticeable on the face and forehead. With the onset of sweating, flushing of the skin occurred, and the respiratory rate rose rapidly. Unlike the dog and cat, there was no panting.

By local heating of the brain at appropriate sites, heat-loss responses, identical to those exhibited by the normal animals, were produced. Palmar and facial sweating were both observed, the former being conspicuous, while the latter was distinct only when its appearance was accentuated by the iodine-starch indicator. When it ensued during heat stimulation of the brain, sweating came on slowly after about 3 min. of heating. During the control intervals of 5 min. or more between the periods of heating, no sweating was observed, though external conditions had not changed. Since perspiration was the most clear-cut response, it was used as the criterion of heat-loss activity. On the projection tracings (Fig. 1) stimulated points have been plotted as negative (dots), slight (open circles) or marked (filled circles) with regard to sweating. The differentiation between slight and marked sweating was of course arbitrary, though in most cases it was easily made.

Accompanying sweating on the heating of reactive regions was a consistent increase in the rate of respiration, an increase which began shortly before the onset of sweating. It is possible that there was minimal, non-detectable perspiration at some points, which may account for the fact that at a few sites, classed as negative, there was a moderate tachypnea. However, comparison of the respiratory rates during excitation of non-responsive points leaves no doubt as to the reality of the augmentation. For 50 negative points, the average respiratory rate before heating the brain was 54 per min., the average high attained during stimulation 59.7 per min., and the mean increase in rate therefore 5.7 per min. For 43 points, the activation of which induced sweating, whether slight or marked, the average beginning rate was 52.7, the high 76.1, and the mean advance, therefore, 23.4 respira-

tions per min. This difference in respiratory rate increment is statistically significant,* and the conclusion that local heating of certain regions of the brain produces polypnea as well as sweating is inescapable.

Though peripheral flushing was at times noted, attempts to measure changes in skin temperature proved largely unsuccessful. Some alterations were observed, but none of truly significant magnitude. It should be noted, however, that neither an ordinary thermocouple nor the Tycos Dermatherm is satisfactory for measuring skin temperature in animals under ordinary laboratory conditions. A radiometer was not available. No noteworthy modifications in rectal temperature attributable to brain heating were remarked. The usual range in rectal temperature during an experiment was from 98.6°F. to 102.0°F. At no time, when positive heat-loss responses were being obtained, were rectal temperatures above 103.0°F.

Localization. Because of the previous delimitation of the heat-sensitive region in the cat, it was not felt necessary to perform an anatomically complete survey of the brain. However, the monkey cerebrum was thoroughly and systematically explored from a level just rostral to that of the genu of the corpus callosum caudally through the tuberal level of the hypothalamus, from the midline to a plane 5 mm. lateral to it, and from the corpus callosum ventrally to the base of the brain. From only one locus within this area were heat-loss reactions induced. A region yielding maximum sweating responses (plotted as solid circles; Fig. 1B) lies in the ventrocaudal part of the telencephalon between the optic chiasma and the anterior commissure. This field has an anteroposterior extent of 1.5 to 2.0 mm., from a level just oral to the crossing of the anterior commissure to the caudal limit of the optic chiasma. The mediolateral spread cannot be fixed exactly, but presumably the sensitive area lies within planes erected parallel to the midline 4 mm. to either side of it, since, when the electrodes were separated by 8 mm. and equidistant from the midline, no sweating was obtained.

Surrounding the area just described, there is a girdle of less marked sensitivity. Rostrally, the first slight sweating responses are found at the very base of the brain, at the level of the diagonal band of Broca (Fig. 1A). Laterally and dorsally to the maximally reactive region slight responses are met with (Fig. 1B), while behind it there are a few scattered minimally responsive points. Also some spots yielding slight sweating are seen ventral

* The average rise in cases of positive sweat response was 23.4 strokes per minute, for negative instances, 5.7. The standard deviation of the mean was computed for each by the formula $\sigma_m = \pm \sqrt{\frac{\sum [m - x_i]^2}{n(n-1)}}$, and found to be 2.09 and 0.968 respectively. The critical

ratio of these two values was determined by the formula $R = \frac{M_2 - M_1}{\sqrt{\sigma_{m_1}^2 + \sigma_{m_2}^2}}$, and ascertained to be 7.65. Since significance is commonly assumed if R is greater than 2, the difference between the accessions of rate is regarded as highly convincing. We are grateful to Dr. F. T. Jung of the Department of Physiology, Northwestern University Medical School, for his suggestions concerning the proper statistical management of these data,

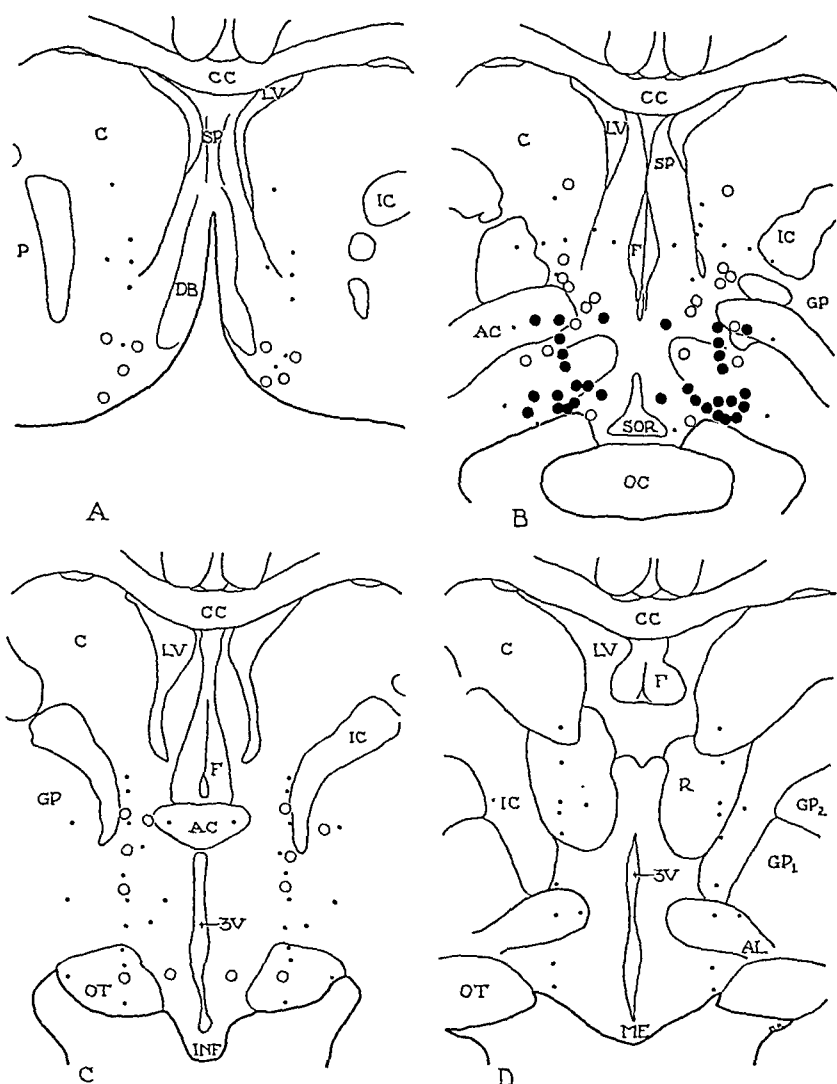


FIG. 1. Four sections at 2 mm. intervals through the preoptic area and anterior hypothalamus of the monkey. Each point on one side of the brain has its counterpart on the other, since all exploration was performed by pairs of electrodes. Symbols: dots, negative; open circles, slight sweating; filled circles, marked sweating. A level one millimeter rostral to that of A showed no positive responses. Abbreviations: AC, anterior commissure; AL, ansa lenticularis; CC, corpus callosum; C, caudate nucleus; DB, diagonal band of Broca; F, fornix; GP, globus pallidus; IC, internal capsule; INF, infundibulum; LV, lateral ventricle; ME, median eminence; OC, optic chiasma; OT, optic tract; P, putamen; R, nucleus reticularis; SOR, supraoptic recess; SP, septum pellucidum.

to the region of unequivocal sensitivity (Fig. 1B and C). The zone from which only slight sweating was elicited is in turn surrounded by areas non-reactive to heat stimulation. At levels 1 mm. rostral to that of Fig. 1A (not illustrated) and 2 mm. caudal to that of Fig. 1C (Fig. 1D), no heat-loss

activity was obtained by local heating. Points lateral and dorsal to the responsive areas are negative (Fig. 1B) as are some points ventral to it (Fig. 1B and C).

DISCUSSION

The strict dependence of the activation of sweating and polypnea on the heating of only a certain portion of the brain and the complete absence of heat-loss behavior during control periods when no current was passing constitute a convincing demonstration of the validity of the observations reported. The inability to measure skin temperature rises is assumed to have been due to inadequate instruments and technique. The periods during which the heat-loss mechanism was activated were too short to cause a fall in body temperature.

Reasons for crediting the results to heat stimulation and not to the severance of any descending pathways from higher centers have been presented elsewhere (Magoun, Harrison, Brobeck and Ranson, 1938). The facts that in most cases there was no extensive destruction of tissue, and that the responses disappeared with the cessation of intracranial heating and were repeatable hold true for the monkey as for the cat and establish emphatic evidence against the surmise that injury might be responsible.

The outcome of these experiments does not prove that the reactive region contains the final efferent supranuclear neurons for the activation of heat-loss mechanisms; it is quite possible that it contains afferent-like elements capable of exciting efferent groups of cells situated more caudally. The interpretation placed on the results is that by artificial heating of the brain the same behavior is made operative as is normally brought into play by a rising cerebral temperature contingent upon an increased degree of warmth in the circulating blood.

The area found to be maximally susceptible to local heating in the monkey coincides closely with the anatomic preoptic region. The band of surrounding lesser responses may represent sites of less compact concentration of the sensitive elements or may illustrate spread of the heat to more specifically reactive points.

The field yielding marked sweating is comparable to that found to be heat-responsive in the cat (Magoun, Harrison, Brobeck and Ranson, 1938). The principal difference between the topographies of response in the two animals is that in the cat a zone of lesser reactivity was encountered through the dorsal hypothalamus; this feature was not present in the macaque. Hemingway, Rasmussen, Wikoff and Rasmussen (1940) have brought forth confirmatory evidence in the dog. They obtained partial activation of heat-loss mechanisms by warming the anterior hypothalamus. Their inability to produce panting was probably due to the fact that the optic chiasma and vascularized scar tissue intervened between the source of heat and the preoptic region. This distance factor must have prevented completely effective heating of the sensitive area. The authors note that a thermocouple placed 2 mm. from the electrode showed body temperature at all times.

Reinforcing the localization afforded by brain heating experiments are the studies of many workers on the results of destructions and transections of the brain-stem; these have been reviewed elsewhere (Ranson, 1940). More concrete affirmation has been given by the consequences of localized lesions in the heat-responsive center in the cat (Teague and Ranson, 1936; Clark, Magoun and Ranson, 1939) showing that its destruction results in impaired ability to regulate against environmental warmth in the chronic state. The results of destroying the heat-sensitive region in the monkey are now being studied (Beaton, McKinley, Magoun and Ranson) and the preliminary experiments confirm the findings of exploration with the high frequency current. When these results are presented the problem of neurogenic hyperthermia will be discussed.

SUMMARY AND CONCLUSIONS

Local heating of the brain of the monkey by a low-voltage, high-frequency current passed between electrodes oriented in the brain with the Horsley-Clarke technique has demonstrated a reactive field which responds by bringing into play the heat-loss mechanisms of sweating and polypnea. The probably associated vasodilatation was not demonstrated because of technical difficulties.

The area in question is located in the preoptic region of the telencephalon between the anterior commissure and the optic chiasma, with a rostro-caudal extent of about 2 mm. and a lateral reach of about 3 mm. to either side of the midline.

The disposition of the heat-sensitive area in the monkey is in close accordance with the location of the similar center previously outlined for the cat. Because the monkey is more closely related phylogenetically to man and because, like man, it sweats but does not pant, the results presented in this paper make it highly probable that the heat-sensitive area has the same location in man. This is in agreement with what little evidence is available from clinical studies.

The results are interpreted as identification of a reactive region which contains elements normally activated by the rising temperature of the blood when the animal is overheated, and in turn activating the mechanisms of heat-loss.

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THE ENDPLATE POTENTIAL DURING AND AFTER THE MUSCLE SPIKE POTENTIAL

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IN THE PRECEDING PAPER (Eccles and Kuffler, 1941) it was shown that a nerve impulse sets up in normal muscle an initial phase of endplate potential (e.p.p.), which is eventually submerged beneath the later spike potential. This initial phase may reach a height as small as 1 per cent or as large as 18 per cent of the peak-potential, but there is much less variation in its rate of rise, which is such that it would attain 30 to 42 per cent (exceptionally 50 per cent) of the peak-potential did the spike not supervene. Once the spike has arisen, the subsequent course of the e.p.p. is lost under the larger spike potential, and, as described in this paper, the following two indirect procedures must be used in determining the remainder of its time course.

(i) In the curarized frog's sartorius the course of the e.p.p. under a muscle spike has already been investigated by studying its interaction with an antidromic volley propagated from a distal point of direct muscle stimulation (Eccles, Katz and Kuffler, 1941b). When the antidromic volley arrives at the endplates synchronously with the setting up of the e.p.p. by a nerve volley, this potential is depressed to about 30 per cent of its height and greatly shortened in duration. If the assumption be made that the e.p.p. is identically affected by an antidromic muscle impulse and by an impulse initiated at the motor endplate, a similar investigation on normal muscle gives the course of the e.p.p. during and after the spike potential set up by a nerve volley. The antidromic impulse would have to be timed so that it arrived at the endplate zone at the instant of initiation of the muscle impulse there, and the course of the e.p.p. would be determined by subtracting the antidromic spike potential from the potential set up by the combined nerve and antidromic stimulation. However this method is only strictly applicable to the isolated single fibre nerve-muscle preparation, which is as yet unobtainable. In the present investigation the soleus strip preparation of the cat and the frog sartorius have been used as imperfect substitutes.

(ii) As shown previously (Eccles and Kuffler, 1941) subparalytic doses of curare diminish the initial phase of e.p.p. preceding the spike; at the same time this diminution also appears as a deficiency of the later part of the action potential at the endplate zone.

Besides determining the course of the e.p.p., this paper also describes excitability changes produced by that part of the e.p.p. which continues during and after the muscle spike potential, and discusses the way in which these effects are produced.

The experimental procedures have already been described (Eccles and O'Connor, 1939c; Eccles, Katz and Kuffler, 1941b). Care must be taken

that the stimulus directly exciting the muscle and setting up the antidromic volley does not also excite motor nerve fibres. With the cat's soleus the innervated strip was usually prepared by aseptic nerve dissection some four days previously, so as to allow time for degeneration of the nerves to the rest of the muscle. Stimulation of the nerve fibres innervating the strip was guarded against by placing the stimulating cathode on the strip at least 6 mm. away from the endplate focus, the anode being about 3 mm. further away.

A. INVESTIGATION BY ANTIDROMIC MUSCLE VOLLEYS (M)

1. *In cat.* In Fig. 1, the recovery of the mechanical response to a nerve volley is shown, following (i) an antidromic volley (crosses— MN series)

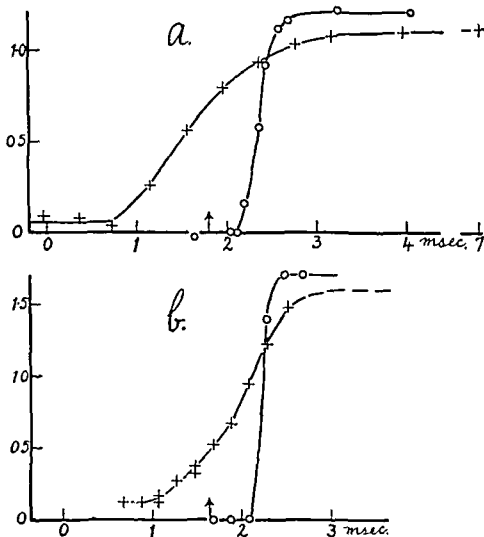


FIG. 1, *a* and *b*. Cat's soleus, two experiments. Contraction added by a nerve volley (N) set up at various times after a preceding (i) antidromic volley (MN series: crosses) or (ii) nerve volley (N_1N_2 series: circles). Ordinates, additional contraction expressed as a fraction of contraction evoked by N alone; abscissae for the N_1N_2 series are the stimulus intervals, and for the MN series the zero interval is chosen as that MN interval at which the M and N spike-peaks are simultaneously at the endplate zone (this spike-peak time indicated by arrow).

and (ii) a preceding nerve volley (circles— N_1N_2 series, cf. Eccles and O'Connor, 1939c, Fig. 10). In order to plot the two curves on a common time scale,

the zero for the MN series is chosen as that MN interval at which the M and N spike-peaks are simultaneous at the endplate zone (see arrow, Fig. 1). This zero for the MN series will be used throughout the paper. The two

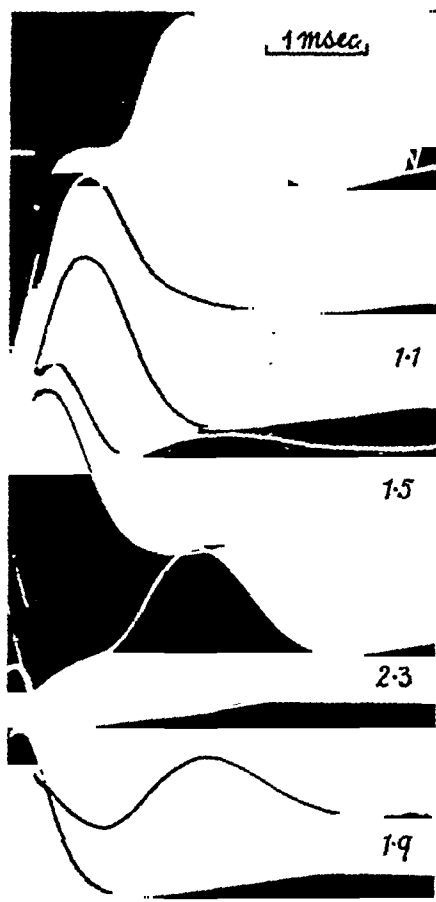


FIG. 2. Endplate zone of innervated strip, cat's soleus. Action potentials added by nerve volley (N) at the indicated intervals in milliseconds after the antidromic volley (M). The base line formed by the M response alone is just below the corresponding MN response. The N stimuli are fired at a constant position on the sweep, and at zero MN interval is chosen so that M and N spike-peaks are simultaneous. Top record, response to N alone, showing position of N stimulus for all MN records.

curves of Fig. 1a or b differ in two respects: (a) at the shortest MN intervals N gives a slight additional contraction, so forming a low initial plateau; and at long volley intervals the MN plateau is lower than the N_1N_2 plateau; (b) with the MN curve the rising phase begins earlier than with the N_1N_2 curve and is at least 1.5 msec. longer.

The difference (a) shows that the M stimulus has failed to excite some fibers of the innervated strip, these being fired off by N , even when simultaneous with M . The initial plateau height indicates that this failure occurred in about 6 and 12 per cent of the fibres in Fig. 1a and b respectively. Even with the most superficial and sharply defined of the innervated strips, it has been impossible to excite directly all their muscle fibres—in seven over 80 per cent were excited—in three over 90 per cent.

(b) The brief rising phase of the N_1N_2 curve indicates that there is very little scatter in the recovery times of the different muscle fibres; hence presumably the much longer duration of the MN rising phase is due to the asynchronism of arrival of the antidromic impulses at their respective endplates. This asynchronism would arise partly on account of differing conduction velocities in the individual muscle fibres, and partly of the differing conduction distances to the endplates, for there is a scatter over about 2 mm. of even a sharp endplate focus.

In Fig. 1 this asynchronism amounts to at least 1.5 msec., for which allowance

must be made in interpreting a series of MN action potentials such as those of Fig. 2. In Fig. 3a the potentials added by N at various MN intervals have

been determined by subtraction. With the shortest *MN* intervals (0.7 and 1.1 msec.) similar potentials are added by the nerve volley, and progressively increasing spikes are shown as the *MN* interval lengthens to 1.5, 1.9 and

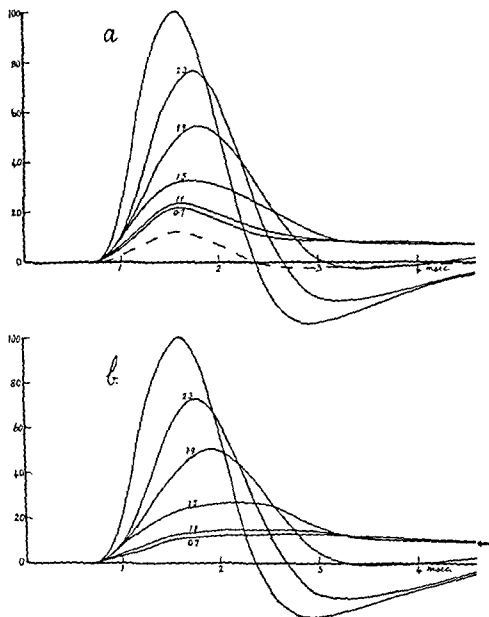


FIG. 3a. Potentials (percentages of normal peak-potential) added by *N* to *M* at the *MN* intervals shown in milliseconds on each curve (cf. fig. 2). The largest potential is response to *N* alone. The broken line is 12% of this *N* response and gives the action potential of the unoccluded fraction of innervated strip (see text). Zero time in this and all subsequent figs. is time of nerve stimulus.

FIG. 3b. As in fig. 3a, but the unoccluded fraction of potential has been subtracted from all curves (see text). The two arrows at extreme right of curves mark the heights of the e.p.p.s with *MN* intervals of 0.2 and 0.4 msec. (see text).

2.3 msec. (cf. this same series in Fig. 1b and 2). The initial peaks in the 0.7 and 1.1 msec. responses indicate that the nerve volley sets up a small spike potential, and the contraction-interval curve (Fig. 1b) shows that this is due to that fraction of the innervated strip (12 per cent) not fired off antidromically. This unoccluded fraction of 12 per cent has been subtracted from all the curves of Fig. 3a to give the potentials set up by *N* in the occluded fraction (88 per cent) of the innervated strip (Fig. 3b). With

the 0.7 and 1.1 msec. intervals N adds a potential closely resembling in size and time course the e.p.p. added by N_2 at intervals of 1.7 and 2.1 msec. in the N_1N_2 series shown in Fig. 4. Thus, when its spike response is prevented by a preceding antidromic volley, a nerve volley sets up an e.p.p., just as it does after the response to a preceding nerve volley.

By the usual matching tests the "e.p.p. response-interval" curve has been plotted in Fig. 5a with circles for the N_2 responses (cf. Fig. 12 and 14, Eccles

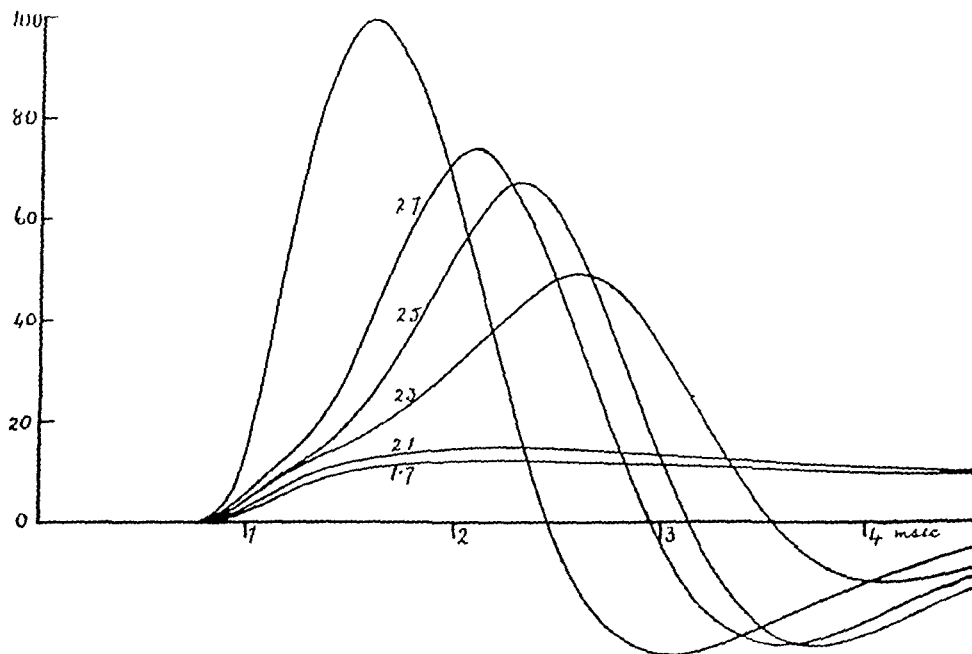


FIG. 4. Experiment of figs. 2 and 4. Potentials (percentages of normal peak-potential) added by N_2 to N_1 at volley intervals indicated in milliseconds on each curve. The largest potential is response to N alone. At N_1N_2 intervals of 1.7 and 2.1 msec. N_2 sets up an e.p.p. only, and an initial e.p.p. step is seen at the three longer intervals.

and Kuffler, 1941). The two upright crosses show the points for the e.p.p.'s set up by N at the MN intervals of 0.7 and 1.1 msec. (cf. Fig. 3b). With longer MN intervals the early origin of the spike prevents any estimate of the endplate potential in Fig. 3b. Moreover, on account of the asynchronism, an antidromic volley, with MN intervals less than 0.7 msec., would fail to reach the last endplates before N had caused them to discharge impulses. This makes it impossible to determine the initial part of the N response by the subtraction analysis. However, that part of the N response beyond the range of such spike interference can be determined and is found to have a time course similar to the e.p.p. set up by N at the 0.7 and 1.1 msec. intervals and to be little if any smaller (cf. arrows to right of Fig. 3b). Assuming that the time courses are also similar over the earlier unanalysable parts, the

maximum potentials may be calculated and are plotted as the two oblique crosses in Fig. 5a.

Figure 5b gives a more complete MN and N_1N_2 series plotted as in Fig. 5a (the N_1N_2 responses have been plotted in Fig. 12, Eccles and Kuffler, 1941). At zero interval, *i.e.*, with simultaneous M and N spikes, N sets up an e.p.p. about 11 per cent of the peak-potential. In three other experiments (cf. Fig. 5a) this e.p.p. has also been about 10 per cent of the peak-potential (respective values: 11, 8.5, 9.5). In our other antidromic experiments an e.p.p. of this order has also been observed for short MN response intervals. It may, therefore, be concluded that at the endplate zone an e.p.p. of about 10

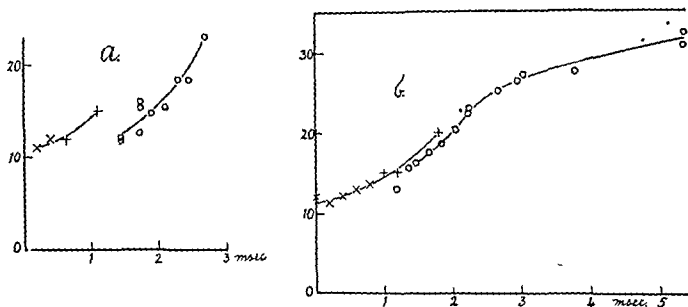


FIG. 5. Ordinates, size of e.p.p. (as percentages of peak-potential) set up by a nerve volley after a previous nerve volley (circles) or antidromic volley (crosses): abscissae, intervals in milliseconds measured as in fig. 1 for N_1N_2 and MN series.

a, experiment partly shown in Figs. 1b, 2, 3 and 4.

b, experiment shown in Figs. 10, 11 and 12 of previous paper (Eccles & Kuffler, 1941).

per cent of the peak-potential is submerged beneath the spike potential set up by a nerve volley.

Normally the rate of rise of the e.p.p. set up by a nerve volley is such that it would attain 30 to 40 per cent of the peak-potential did the spike not supervene (Eccles and Kuffler, 1941). During the refractory period set up by an antidromic volley this maximum is diminished to about 10 per cent of the peak-potential, *i.e.*, to about 30 per cent of its normal value. It is of interest that the antidromic volley has an almost identical effect on the e.p.p. in completely curarized muscle (Eccles, Katz and Kuffler, 1941b).

The MN series of Fig. 5a and b show the increasing e.p.p. set up by N as the MN interval lengthens, *i.e.*, as tested by the e.p.p. response, there is a progressive recovery from the refractoriness. In both figures there is a suggestion that with the N_1N_2 series, the e.p.p. set up by N_2 recovers along a slightly lower curve; however, these figures certainly indicate that the refractoriness of the muscle is largely responsible for the diminished e.p.p.'s

set up by N_2 at short N_1N_2 intervals. The closeness of the N_1N_2 and MN curves in Fig. 5b also suggests that the preceding N_1 produces no large diminution in the exciting power of N_2 , until the N_1N_2 interval is less than 1.5 msec. This is also indicated by the e.p.p.'s set up by N_2 at various intervals in completely curarized muscle (cf. Eccles, Katz and Kuffler, 1941b, Fig. 17a).

The antidromic investigation has given the approximate size and time course of the e.p.p. which underlies the spike potential set up by a nerve volley; hence the total action potential set up by a nerve volley at the endplate zone may be separated into its component spike and e.p.p. Fig. 6a shows such an analysis for the simple-spike potential set up by a nerve volley in Fig. 2. When there is a large initial e.p.p. step (cf. Fig. 1, 4 and 10, Eccles and Kuffler, 1941), the membrane breakdown associated with the spike presumably results in a diminution of this potential, for the initial step may actually be greater than the maximum e.p.p. added on top of the spike. Some indication of the speed of this diminution (see dotted line in Fig. 6b) is given by the action of the antidromic volley on the decay of the e.p.p. in curarized muscle (cf. Eccles, Katz and Kuffler, 1941b).

2. *In frog's sartorius*. Frog's sartorius is in some respects more suitable for investigating the interaction between M and N , especially as complete occlusion can be achieved by

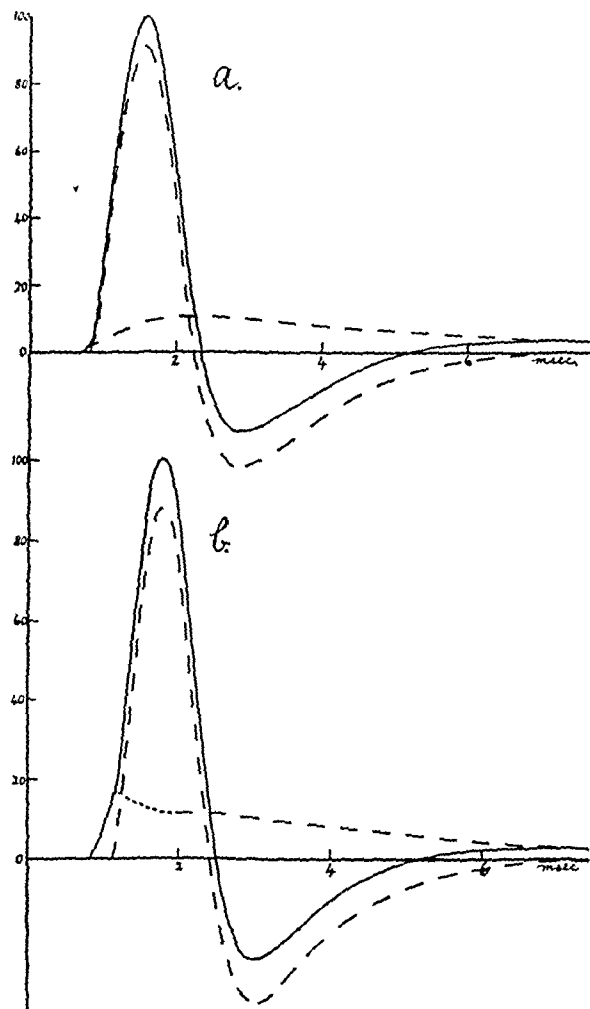


FIG. 6. Continuous line shows action potential set up at endplate zone by a nerve volley. Lower broken line shows e.p.p. component of this potential as determined by antidromic experiments. Upper broken line shows spike potential as determined by subtracting e.p.p. from total potential. Ordinates, potentials as percentages of peak potential: abscissae, time after nerve stimulus.

a, experiment of figs. 1b, 2, 3, 4, 5a.

b, experiment of fig. 5b.

maximal stimulation of all the muscle fibres.

The e.p.p.'s added by the testing nerve volley are determined by subtraction as in Fig. 3a and 4, and MN intervals are measured as above so as to be comparable with the N_1N_2 intervals. With similar short MN and N_1N_2 intervals (1.0–1.5 msec., 23°C.) the added e.p.p.'s do not differ significantly (cf. Fig. 5b). However, as the MN interval was still further shortened, the e.p.p. was much diminished and decayed more rapidly. With zero MN interval it was only about 40–50 per cent of that set up by N_2 at N_1N_2 intervals of 1.0–1.5 msec., and little survived the antidromic spike potential. Thus it may be concluded that the frog's sartorius resembles the cat's soleus in that an e.p.p. is submerged beneath the spike potential set up by a nerve volley, but it appears to be smaller and very little survives the spike potential.

B. INVESTIGATION BY CURARIZATION

1. EFFECT ON ACTION POTENTIAL SET UP BY A SINGLE NERVE VOLLEY

Subparalytic curarization diminishes the rate of rise of that part of the e.p.p. which precedes the spike origin (Eccles and Kuffler, 1941). At the same time there is always a diminution in the negativity at the endplate zone for some time after the spike potential (cf. Fig. 13a), and in Fig. 7a and b, curve *ii*, the time course of this diminution has been determined as the difference between the potentials set up at the endplate zone by a single nerve volley before and after subparalytic curarization. Beyond 6 msec. the time course of decay resembles that for the e.p.p. set up by a second nerve volley in the normal muscle (curve *i*), and at shorter intervals the deviation is explicable by the delay of the spike potential produced by subparalytic curarization (cf. Eccles and Kuffler, 1941, Fig. 4). Hence this deficient negativity is due to a diminution by curare of that part of the e.p.p. which normally outlasts the spike potential set up by a single nerve volley.

In normal muscle a deficiency with a similar time course is shown for the response to a second nerve volley at intervals from 20 to 200 msec. after the first volley. Usually this deficiency is about 20 per cent of the e.p.p. set up by an early second nerve volley, but in curve *iii*, Fig. 7b, it is as much as 50 per cent (curve *i*). Curves *iv*, Fig. 7a and b, show that the depression of the second volley and subparalytic curarization are cumulative in producing a deficiency of e.p.p.

2. EFFECT ON ACTION POTENTIAL SET UP BY AN EARLY SECOND NERVE VOLLEY

Figure 8a shows typically that subparalytic curarization not only diminishes the e.p.p. added by an early second nerve volley, but also quickens its rate of decay. Normally (curve *i*) this rate is slower than with the pure e.p.p. of completely curarized muscle (curve *iv*), but, with a depth of curarization just less than partially paralytic (curve *iii*), there is considerably faster decay than in complete curarization. However, plotting semi-logarithmically (Fig. 8b) shows that with all depths of curarization the later parts of the e.p.p.'s (from about one half decay onwards) are approximately parallel straight lines, i.e., all have practically the same rate of exponential

decay. With curarized muscle this phase of decay is due to passive dissipation of membrane potential (Eccles, Katz and Kuffler, 1941b); hence the present experiments show that curare has no appreciable action on the electric constants of the muscle membrane. The initial slow rate of decay with normal muscle must be due to the persistent action of some depolarizing agent—

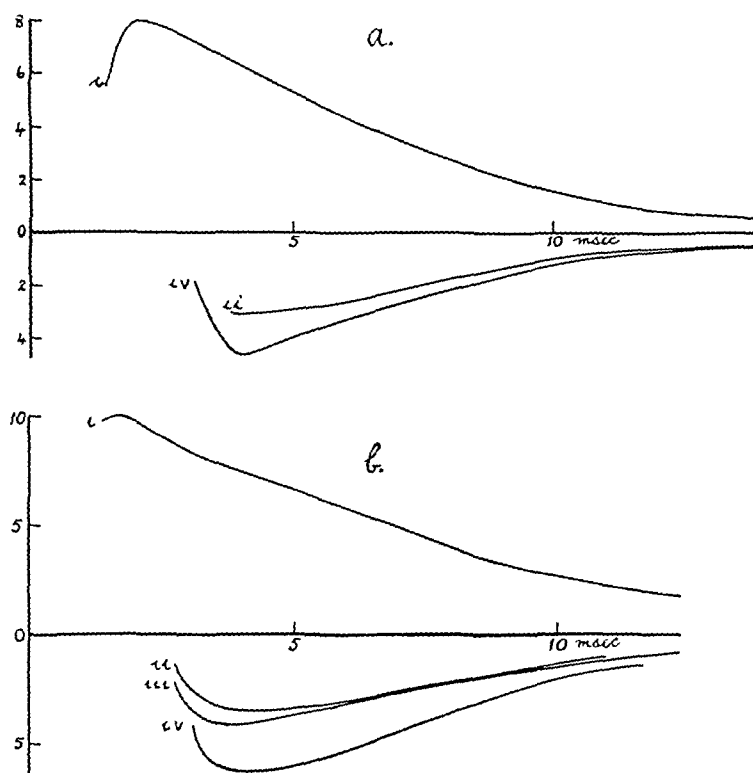


FIG. 7. *a* and *b* show two experiments on cat's soleus with recording electrode on end-plate zone of innervated strip. Ordinates, percentages of peak-potential; abscissae, time in milliseconds from stimulus. Curve *i* is the e.p.p. added by a second nerve volley 1.6 msec. after the first, zero being time of N_2 stimulus. Continuous line below base line is deficiency which subparalytic curarization produces in action potential set up by a single nerve volley. Curve *ii* shows deficiency (relative to normal single response) of action potential set up by a second nerve volley 24 msec. after first. Curve *iv* shows deficiency (relative to normal single response) of action potential set up in subparalytically curarized muscle by second nerve volley 24 msec. after first. Zero time for curves below base line is time of nerve stimulus.

presumably that acting initially and producing the e.p.p. Thus subparalytic curarization not only diminishes the effect produced by this depolarizing agent (cf. Eccles and Kuffler, 1941, section A1), but it also shortens its duration of action. Such shortening of action will not account for an initial decay faster than with the completely curarized response (cf. curves *iii* and *iv*, Fig. 8), for, if anything, the action should then be still shorter. However, it has been shown that, on account of diminished membrane impedance, the

e.p.p. decays much more rapidly in refractory muscle (Eccles, Katz and Kuffler, 1941), and presumably this is the explanation of the rapid initial rates of decay with the deepest subparalytic curarizations. Thus we have two opposing factors normally modifying the decay of the e.p.p. set up by an early second nerve volley: (i) the refractoriness resulting from the muscle

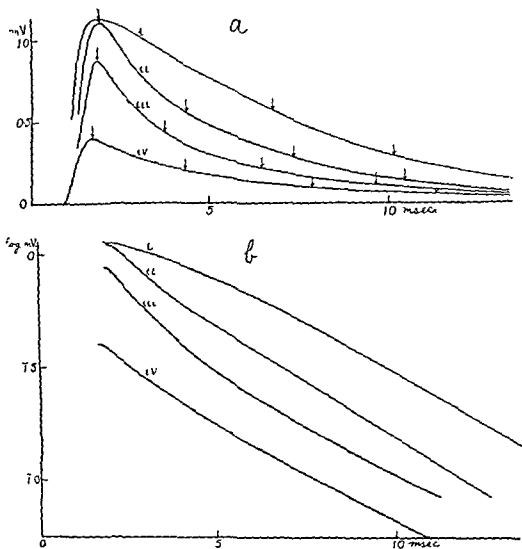


FIG. 8a. Cat's soleus, endplate zone of innervated strip. Ordinates, potentials in mV.; abscissae, time in milliseconds from nerve stimulus. Upper three curves show e.p.p. set up by N_2 1.6 msec. after N_1 : i, normal muscle; ii and iii, subparalytic curarization; iv, e.p.p. set up by a single nerve volley in complete curarization. Arrows mark summits and times of half, quarter and eighth decay of e.p.p.

FIG. 8b. As in fig. 8a, but ordinates are logarithms of potentials. Only decaying phases of e.p.p.'s are plotted.

spike response to the first nerve volley tends to make the rate of decay greater during the first 6 msec., and (ii) the depolarizing agent responsible for setting up the e.p.p. continues to exert its active depolarizing effect for about 6 msec. This latter action is normally more powerful than the former, so initially the e.p.p. decays more slowly than in completely curarized muscle; with subparalytic curarization the situation is reversed.

The antidromic experiments (section A) have shown that the e.p.p.'s

set up by a single nerve volley and by an early second nerve volley decay similarly (cf. Fig. 3b and 4); hence the above two factors also presumably condition the decay of the e.p.p. set up by a single nerve volley in normal muscle. The persistent action exerted on the muscle membrane by the depolarizing agent accounts for the normal e.p.p. outlasting the spike potential in much the same way as the e.p.p. set up by an early second nerve volley. On the other hand it has been shown that in curarized muscle the depolarizing action is very transient and practically no e.p.p. then outlasts the rapid period of decay that occurs during a muscle spike (Eccles, Katz and Kuffler, 1941b). Even with subparalytic curarization, curve *iii*, Fig. 8a, suggests that, in the response to a *single* nerve volley, very little e.p.p. would outlast the muscle spike. Therefore subtraction of such a subparalytic potential from the normal response to a single nerve volley should give nearly the full normal e.p.p. for the region after the spike. Thus, for example, in Fig. 7a the e.p.p. of curve *ii* is 65 per cent of the e.p.p. set up by an early second nerve volley (curve *i*), and curve *iv* is actually 80 per cent of curve *i*. Therefore in its later part, at least, the e.p.p. set up by a single nerve volley must be at least 80 per cent of that set up by an early second nerve volley, a result in agreement with the antidromic experiments (cf. Fig. 5a and b).

Normally the frog's sartorius resembles the subparalytically curarized cat's soleus, in that very little e.p.p. survives the spike potential set up by a single nerve volley (Section A2); this suggests that the depolarizing action is briefer.

C. EFFECTS PRODUCED BY THE ACTION OF THE ENDPLATE POTENTIAL DURING AND AFTER THE MUSCLE SPIKE

In sections A and B it has been shown that part of the e.p.p. set up by a nerve volley persists during and after the muscle spike also set up by this volley. It will be seen (section 1) that this concurrent e.p.p. lengthens the minimum interval at which a later "testing" nerve volley sets up a muscle spike—"the least spike-interval," *i.e.*, the e.p.p. delays recovery from the muscle's refractory period. In addition it will be seen (Section 2) that the concurrent e.p.p. raises the threshold e.p.p. at which the spike is initiated.

1. LENGTHENING OF LEAST SPIKE-INTERVAL BY THE ENDPLATE POTENTIAL

The following five investigations are designed to give varying sizes and time courses of the e.p.p. during and after a muscle spike, and the effect of this e.p.p. is tested by the response to a later nerve volley. As this testing nerve volley is always more than 1.5 msec. after the immediately preceding nerve volley, no serious error is introduced by neglecting any diminution which this preceding volley may produce in its excitatory power (cf. Section A1). With short volley intervals the preceding nerve volley lengthens the latency of the e.p.p. set up by the testing nerve volley, but with intervals beyond 1.5 msec. this is no more than 0.1 msec. (cf. Fig. 22, Eccles and O'Connor, 1939c) and may be neglected when considering the much greater lengthenings of spike latency.

a. Comparison of MN with N_1N_2 series. In Fig. 9 the ordinates are the latent periods of the muscle spike-peaks set up by the testing nerve stimulus, and abscissae are the time intervals between the M and N or the N_1 and N_2 stimuli brought to a common time scale as in Fig. 1 above. As the testing N stimulus is moved closer to the initial spike-peak (shown by arrow in Fig. 9) there is a lengthening of the spike's latent period with both the N_1N_2 and MN series. With the MN series the curve eventually rises at 45° , showing that the latent period is lengthened by an amount equal to the shortening of the MN interval, i.e., the intervals between the M and N spike-peaks reach a constant value, which the intersection of the 45° line with the base line shows to be 2.15 msec. The 3 points for the N_1N_2 series in Fig. 9 give the least spike-interval for the N_1N_2 series as 3.15 msec., again by the intersection of the 45° line with the base line. Thus Fig. 9 shows that the least spike-interval is lengthened from 2.15 msec. to 3.15 msec. when an e.p.p. is present during the refractory period. Table 1, column 4, shows the lengthenings of least spike-intervals in the 8 experiments in which this investigation has been carried out, the average being 0.83 msec. with extremes of 0.6 and 1.1 msec.

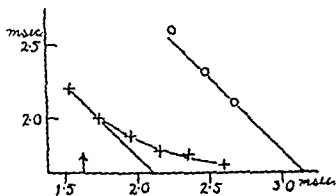


FIG. 9. Cat's soleus, endplate zone. Ordinates, latent periods of muscle spike-peaks set up by testing nerve stimulus at various intervals: abscissae are stimulus intervals with the N_1N_2 series (circles—cf. fig. 4), and with the MN series (crosses—partly shown in figs. 2 and 3); zero interval is chosen so that M and N_1 spikes synchronize at the arrow (cf. fig. 1). Base line is drawn at normal latent period for spike set up by N , hence intersections with 45° lines give the least spike-intervals for the MN and N_1N_2 series.

It has been observed that sometimes there is supernormal conduction of the earliest muscle impulses set up by a second nerve volley (Eccles and O'Connor, 1939c, p. 95). This suggests that recovery from refractory period is slower at the endplate zone than elsewhere in the muscle, an effect attributable at least partly to the e.p.p.

b. Comparison of MnN series with the MN and N_1N_2 series. This relationship of e.p.p. to lengthening of the least spike interval has been further investigated in two antidromic experiments by interpolating a conditioning nerve volley (n) between the antidromic volley, M , and the testing nerve volley, N . In Fig. 10 several such series of observations for different Mn intervals are plotted as in Fig. 9, together with the MN and N_1N_2 curves. When n is timed so that its spike response would approximately coincide with the antidromic spike, it lengthens the spike-intervals so that the recovery curve approximates to that for N_1N_2 (cf. the M 0.1 msec. n curve in Fig. 10), as would be expected if n added the same e.p.p. to the antidromic spike that it would normally add to its own muscle spike response. This supports the original assumption at the beginning of this paper, which

Table 1. Table showing in columns 2, 3, 6 and 8 the least spike-intervals in milliseconds for the various series of stimuli described in Section C1

Experiment	Section C1a: MN, N_1N_2			Section C1b: MnN		Section C1c: N_1nN_2		
	MN least spike intervals	N_1N_2 least spike intervals	Length- ening: column 3—col- umn 2	Mn intervals	Least spike intervals	N_1n intervals	Least spike intervals	Average length- ening by n : column 8—col- umn 3 9
1	2	3	4	5	6	7	8	9
2/11/38		2.6				0.8 1.2 1.4 1.6 1.8 2.0	3.65 3.65 3.7 3.8 3.4 3.5	1.0
8/11/38		2.8				1.6 1.9	4.3 3.8	1.15
11/11/38		3.0				1.0 1.4 1.8	3.85 3.85 4.25	1.0
24/ 2/39		2.6				1.0 1.4 1.8	4.0 4.2 4.1	1.5
2/ 3/39		3.0				1.1 1.7	4.3 4.0	1.15
4/ 8/39		2.55				1.15	3.8	1.25
18/ 8/39	2.1	3.0	0.9	0 0.15 0.3	3.0 3.3 3.6			
12/ 9/39	2.1	2.8	0.7					
15/ 9/39	2.0	3.1	1.1					
27/10/39	2.8	3.4	0.6	0.1 0.45 0.95 1.3 1.7	3.3 3.6 4.05 4.35 4.6	1.2 1.9	5.2 5.4	1.9
4/12/39	2.2	2.85	0.65					
18/ 4/40	2.2	3.0	0.8					
4/ 7/40	2.15	3.15	1.0					
20/ 9/40	2.2	3.05	0.85					
Average lengthenings			0.83					1.28

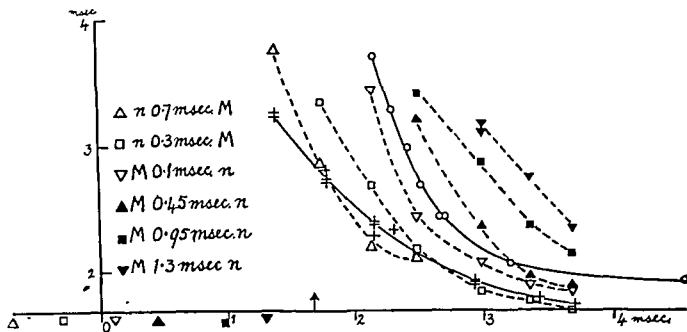


FIG. 10. MnN series. MN (crosses) and N_1N_2 (open circles) plotted as in fig. 9 (another experiment), but 45° lines not drawn. In addition a conditioning nerve volley, n , has been set up at six different intervals before or after M , and the corresponding six series of points with their broken-line curves give the latencies of the spike-peaks set up by the later testing nerve volley. The Mn intervals are shown by different symbols and the time of the n stimulus relative to the M (at zero time) is indicated by the corresponding symbol below the base line. The arrow shows time of M spike-peak.

formed the basis of the determination of the e.p.p. set up normally by a single nerve volley (Section A). With n later relative to M , i.e. with n setting up its e.p.p. some time after the beginning of the antidromic spike, Fig. 10 shows that the spike-intervals are still further lengthened. Thus, with the interpolated e.p.p. beginning 0.45, 0.95 and 1.3 msec. after the antidromic spike, the respective least spike-intervals are lengthened by 0.8, 1.25 and 1.55, as against a lengthening of only 0.6 msec. for the N_1N_2

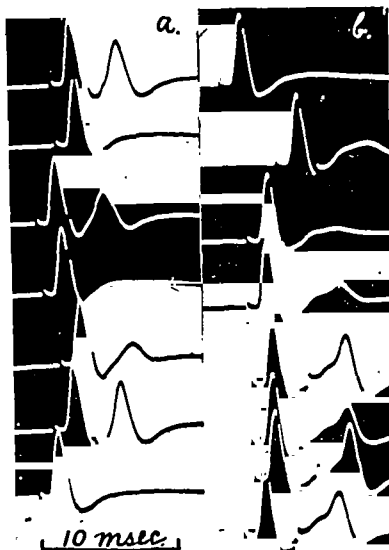


FIG. 11. Same experiment as fig. 10.

a. N_1N_2 series: intervals from top down in milliseconds: 2.8, 1.8, 2.55, 2.15, 2.4, 3.2; lowest record N_1 alone.

b. N_1nN_2 series: N_1n constant at 1.9 msec: top record, N_1n ; remainder, N_1nN_2 series at N_1N_2 intervals from top down in milliseconds: 3.1, 2.7, 3.5, 4.3, 3.9, 4.7, 4.05.

curve. Two factors would give this effect: (i) the later the interpolated e.p.p., the larger it would be (Fig. 5); (ii) the later the e.p.p. the longer it would survive, and hence the more effective it would be in prolonging the refractory period.

When the n volley is so early relative to M that it sets up its muscle spike before M , the latent period curve should be identical with the N_1N_2 curve, for the antidromic volley (M) is blocked from reaching the endplates. With the plotting of Fig. 10 such curves would be N_1N_2 curves shifted to the left by the time the n spike precedes the M spike and this is seen for the n 0.7 msec. M curve, but with the n 0.3 msec. M curve, the shift is 0.1 msec. more than would be expected, and, with M 0.1 msec. n , the curve is 0.2 msec. earlier.

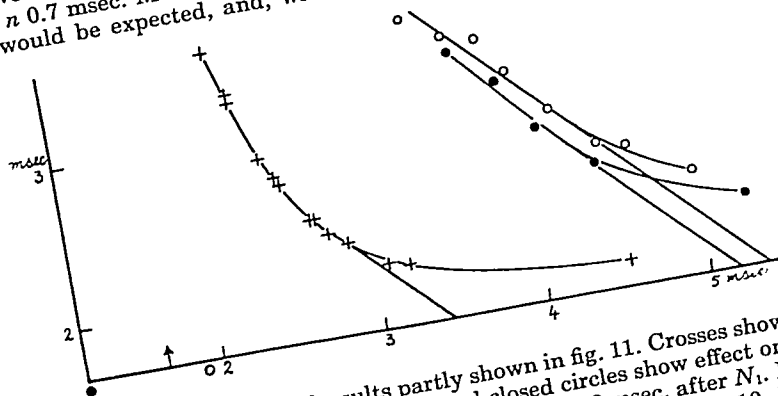


FIG. 12. Plotting as in fig. 9 of results partly shown in fig. 11. Crosses show N_1N_2 curve (cf. N_1N_2 curve open circles, of fig. 10). Open and closed circles show effect on spike latent periods of conditioning volley, n , 1.9 msec. (fig. 11b) and 1.2 msec. after N_1 . Positions of n relative to N_1 shown by closed and open circles below base line as in fig. 10. N_1 stimulus is at zero on scale and arrow shows time of its spike-peak.

Possibly this shift is partly due to the muscle spike being speeded up by the concurrence of two excitatory mechanisms—the eddy currents preceding the propagating antidromic volley and the depolarizing effect of the neuromuscular transmitter. In curarized muscle the e.p.p. was similarly observed to produce a slight speeding (0.1 to 0.2 msec.) of the antidromic volley (Eccles, Katz & Kuffler, 1941 b.).

c. *Comparison of N_1nN_2 with N_1N_2 .* An early second nerve volley (n) adds its e.p.p. to that of the first volley (N_1) without setting up a spike potential (cf. Fig. 4), and, correspondingly, comparison of Figs. 11a and b shows that there is a lengthening of the least spike-interval as tested by a third nerve volley (N_2). This is the "brief depressant action" described by Eccles and O'Connor (1939a). In Fig. 12 the interpolated nerve volley, n , at 1.2 or 1.9 msec. after N_1 lengthens the least spike-interval as much as 2 msec. The above factors *i* and *ii* of section *b* might account for this lengthening being so much greater than the 0.6 msec. lengthening produced by the e.p.p. of a single nerve volley acting synchronously with the muscle spike (comparison of N_1N_2 and MN curves in Fig. 10).

However Columns 7 and 8, Table I, show that there are limits to the action of these factors, for there is no significant relationship between the N_1n interval and the lengthening of the least spike-interval. But, for very short N_1n intervals, n sets up an e.p.p. at a constant interval of about 1.4 msec. after the N_1 response (Eccles and O'Connor, 1939c, p. 77); hence these observations of Table 1 are not inconsistent with those in columns 5 and 6 (MnN series, cf. Fig. 10).

d. N_1N_2 before and after subparalytic curarization. The superimposed records of Fig. 13a show the diminution which a subparalytic dose of curare produces in the e.p.p. that survives the spike (cf. Section B1). Associated with this diminution there is a shortening of the least spike-interval (Fig. 13c and d), the spike arising much earlier on the course of the e.p.p. set up by N_2 . The diminution of e.p.p. produced by subparalytic curare has always (seven experiments) been associated with a diminution in the least spike-interval.

Fig 13.

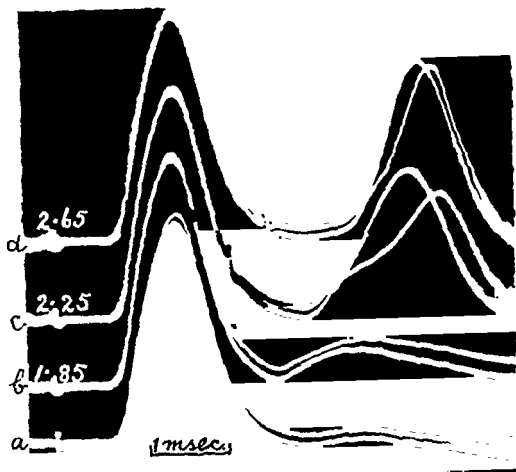


FIG. 13. Endplate zone, cat soleus; a, b, c and d each show superimposed records of action potentials set up before and after subparalytic curarization: a, single nerve volley; b, c and d, two nerve volleys at intervals indicated in milliseconds. After the spike curare is seen to produce a deficiency of the action potential set up by a single nerve volley (see a, and also b, c and d before response to 2nd volley).

This diminution has varied from 0.3 to 0.5 msec., and so is about half the difference between the N_1N_2 and MN least spike-intervals (Table 1, column 4). Thus in subparalytic curarization there is good agreement between this diminution of least spike-interval and the diminution of e.p.p., which is also about halved (column 3, Table 1, Eccles and Kuffler, 1941).

e. N_1N_2 before and after eserinizization. Eserine increases and prolongs the e.p.p. set up by a single nerve volley (Eccles and O'Connor, 1939b; Eccles, Katz and Kuffler, 1941a), and correspondingly there is a lengthening of the least spike-interval. With increasing dosage the e.p.p. and the least spike-interval increase *pari passu*, until a maximum effect is attained with about

0.5 mg. per kg. Table 2 shows that this maximum lengthening of least spike interval averaged 2.0 msec. In four of these experiments eserine was shown (cf. Table 2, *MN* series) to have no significant action on the least spike-interval when the testing nerve volley follows an antidromic volley, *i.e.* it has no action on the true refractory period of the muscle. The action of eserine will be fully described in a later paper.

Table 2. Table showing least spike-intervals in milliseconds for *MN* and N_1N_2 series normally and after a dose of eserine sufficient to give the maximum lengthening with the N_1N_2 series (cf. Section C1e)

Experiment	<i>MN</i> series		N_1N_2 series		
	Normally	After eserine	Normally	After eserine	Lengthening
4/10/38			3.1	5.4	2.3
25/10/38			3.3	5.6	2.3
24/ 7/39			3.0	6.0	3.0
4/ 8/39			2.55	4.3	1.75
18/ 8/39	2.1	2.15	3.0	5.0	2.0
12/ 9/39	2.1	2.1	2.8	4.2	1.4
15/ 9/39	2.0	2.2	3.1	4.6	1.5
11/10/39			3.2	5.1	1.9
27/10/39	2.8	2.85	3.4	5.1	1.7

Discussion

The above evidence shows that the muscle's refractory period is lengthened by concurrent e.p.p., and, when this is large, the least spike-interval may even be doubled (cf. three experiments of Table 2). Now e.p.p. is a cathodal polarization of the junctional region of the muscle (Eccles, Katz and Kuffler, 1941b); hence these observations are related to those of Blair and Erlanger (1933, p. 546), who find that a nerve's refractory period is lengthened by a brief intercurrent cathodal polarization (cf. also Bugnard and Hill, 1935).

2. THE THRESHOLD E.P.P. FOR INITIATING A SPIKE

When the normal action potential has a large initial e.p.p. step (12–18 per cent of the peak-potential), it is probable that this e.p.p. is solely responsible for setting up the muscle impulse, *i.e.* the spike potential (Eccles and Kuffler, 1941). The time course of the e.p.p. during and after the spike can be determined as in Section A (cf. Fig. 6); hence it is possible to construct the time courses of the total e.p.p. set up by two nerve volleys at various short intervals apart. This has been done in Fig. 14a for a double-step experiment in which the spike arose when the initial e.p.p. was 18 per cent of the peak-potential. The e.p.p. set up by each second nerve volley (N_2) is added on to the background e.p.p. of N_1 , and the point of spike origin is ringed by a circle. The summed e.p.p. curve is thereafter concealed under the spike and so can be continued no further. Through these spike origins of Fig. 14a a curve may be drawn, which gives the threshold e.p.p. as determined by N_2

at various times after the muscle's response to N_1 . At the shortest interval this threshold is more than 50 per cent above the normal threshold, and with lengthening N_1N_2 intervals it declines at first rapidly, then more slowly, being still considerably above threshold with N_2 6 msec. after N_1 .

However the responses at still shorter N_1N_2 intervals (Fig. 14b) show that N_2 does not merely test the e.p.p. threshold at various times after N_1 , but

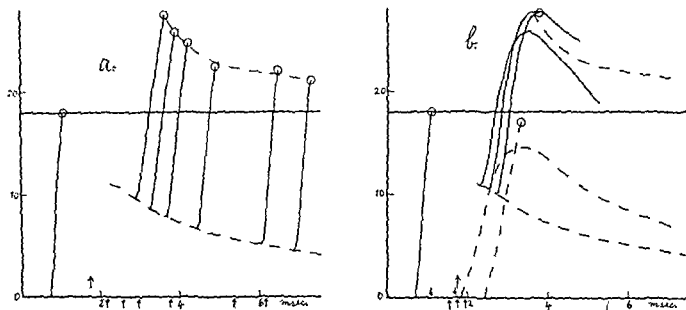


FIG. 14a. Endplate zone, cat soleus, N_1N_2 series (experiment of figs. 10 & 11, Eccles & Kuffler, 1941). Ordinates, e.p.p. as percentage of peak-potential; abscissae, time after N_1 stimulus. To left the initial e.p.p. is shown rising steeply and setting up a spike at the normal threshold e.p.p. (18%). Arrow pointing upwards above base line gives time of spike-peak. Lower broken line gives subsequent course of N_1 e.p.p. as determined by antidromic experiment. On top of this is shown the e.p.p. added by N_2 at the six N_1N_2 intervals shown by arrows below the base line. For each the e.p.p. begins about 0.7 msec. later and rises to set up a spike at point ringed by circle. Upper broken line shows e.p.p. threshold curve.

FIG. 14b. As in fig. 14a, but for the three shorter N_1N_2 intervals shown by arrows below base line. The continuous lines show the corresponding e.p.p.'s added on to the background N_1 e.p.p., a spike being set up only at longest N_1N_2 interval. Arrows pointing downwards above base line show two positions of N stimulus in corresponding MN series, the M spike-peak being synchronous (at large arrow) with that for N_1 of N_1N_2 series (cf. fig. 1). The two broken lines beginning about 0.7 msec. later than the N stimuli show courses of e.p.p. set up by N , the spike origin with M 1.85 msec. N being ringed by the circle (further description in text).

it also conditions this threshold to some extent. Thus with N_1 1.75 msec. N_2 the summed e.p.p. goes well above the threshold curve of Fig. 14a, and yet sets up no spike, while at N_1 1.95 msec. N_2 , a spike is not initiated till about 0.15 msec. after the summed e.p.p. has crossed the threshold curve, i.e. shortening the N_1N_2 interval from 2.15 msec. to 1.95 msec. lengthens the spike-interval by 0.15 msec. This effect is always observed, and has already been described as an example of the Lucas C type of curve (Eccles and O'Connor, 1939c, p. 79). Presumably N_2 raises the e.p.p. threshold in this way on account of the e.p.p. which it adds to the background e.p.p. of N_1 . With the longer volley intervals Fig. 14a shows that the e.p.p. set up by N_2

0.5 mg. per kg. Table 2 shows that this maximum lengthening of least spike interval averaged 2.0 msec. In four of these experiments eserine was shown (cf. Table 2, *MN* series) to have no significant action on the least spike-interval when the testing nerve volley follows an antidromic volley, *i.e.* it has no action on the true refractory period of the muscle. The action of eserine will be fully described in a later paper.

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	Normally	After eserine	Normally	After eserine	Lengthening
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24/ 7/39			3.0	6.0	3.0
4/ 8/39			2.55	4.3	1.75
18/ 8/39	2.1	2.15	3.0	5.0	2.0
12/ 9/39	2.1	2.1	2.8	4.2	1.4
15/ 9/39	2.0	2.2	3.1	4.6	1.5
11/10/39			3.2	5.1	1.9
27/10/39	2.8	2.85	3.4	5.1	1.7

Discussion

The above evidence shows that the muscle's refractory period is lengthened by concurrent e.p.p., and, when this is large, the least spike-interval may even be doubled (cf. three experiments of Table 2). Now e.p.p. is a cathodal polarization of the junctional region of the muscle (Eccles, Katz and Kuffler, 1941b); hence these observations are related to those of Blair and Erlanger (1933, p. 546), who find that a nerve's refractory period is lengthened by a brief intercurrent cathodal polarization (cf. also Bugnard and Hill, 1935).

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solely responsible for initiating impulses. A similar transition is observed as muscle is progressively curarized (cf. Eccles and Kuffler, 1941).

In Fig. 14b the e.p.p.'s set up by a nerve volley (N) at various times after an antidromic volley (M) are plotted as broken lines. As the MN interval is lengthened, N sets up a progressively larger e.p.p. (the e.p.p. at zero MN interval is shown by the lower broken line), and a spike is initiated when this e.p.p. approximately attains the normal threshold value, *i.e.* there is no raised e.p.p. threshold as with the N_1N_2 series. The MN and N_1N_2 series similarly differ in simple-spike experiments such as Fig. 3b and 4. The e.p.p. set up by N_1 must, therefore, be responsible for the raised e.p.p. threshold for N_2 in Fig. 14 and 15. On the other hand the diminished production of e.p.p. by the testing nerve volley is a result of the muscle's refractory period, for it is similarly observed with both the MN and N_1N_2 series (cf. Fig. 3b, 4 and 5).

Further evidence that the threshold e.p.p. is raised and recovery delayed by e.p.p. acting during the refractory period has already been given in Sections C1 c, d and e. The effect of subparalytic curarization is of particular interest in this respect. Not only is the least spike-interval shortened (Fig. 13c and d, Section C1, d) and the threshold e.p.p. diminished on account of the smaller e.p.p. set up by N_1 , but an early N_2 may actually add a larger e.p.p. than it does normally (cf. Fig. 13b at N_1 1.85 msec. N_2 where the added e.p.p. is initially larger, but decays rapidly). This latter effect is exceptional, but with subparalytic curarization the e.p.p. set up by an early N_2 volley is always much less diminished than that set up by N_1 . Possibly, by delaying the muscle's recovery, the e.p.p. of N_1 normally diminishes the e.p.p. produced by a given N_2 , and this effect becomes smaller as the e.p.p. of N_1 is diminished by curare, thus counteracting to some extent the direct depressant action of curare on the N_2 response. Such an effect is also indicated by the slightly higher recovery curves observed for the e.p.p. in the MN as compared with the N_1N_2 curves of Fig. 5a and b.

3. DISCUSSION

It has been shown above and by Eccles, Katz and Kuffler, (1941b) that, in the true refractory period of the muscle set up by an antidromic volley, there is a diminished polarizability of the muscle at the endplate region, for the neuro-muscular transmitter sets up a diminished e.p.p. E.p.p. acting during the refractory period gives an additional depressant action—the raised e.p.p. threshold for initiating impulses. Hodgkin (1938, p. 107) found that the polarizability of the nerve membrane was diminished to less than half during the spike peak. His results, *e.g.* Fig. 14, also indicate that the threshold local potential for spike initiation is higher in refractory nerve, and this may well have occurred in our antidromic experiments at intervals too short for the nerve volley to test the e.p.p. threshold.

A later paper will describe the repetitive discharge which the large and prolonged e.p.p. sets up in eserinated muscle, and sometimes in normal muscle.

SUMMARY

Two experimental procedures have been used in determining the size and time course of the endplate potential, e.p.p., during and after the muscle spike potential set up by a single nerve volley.

(i) The potential added by a nerve volley on top of the antidromic spike potential set up by direct stimulation.

(ii) The effect of subparalytic curarization.

It is thus shown that in the cat the e.p.p. attains a maximum of about 10 per cent of the peak-potential and has a time course similar to the e.p.p. added by an early second nerve volley. Two opposing factors modify this time course, the first being normally the stronger: (a) the depolarizing action of the neuro-muscular transmitter continues normally for about 6 msec.; (b) during the muscle's refractory period (up to 6 msec.) the e.p.p. decays more rapidly.

In the frog much less e.p.p. outlasts the spike, suggesting that factor a is briefer than in the cat.

In the cat the e.p.p. coexisting with the muscle spike has been shown to have two actions.

i. It prolongs the muscle's refractory period, lengthening by about 0.81 msec. the least spike-interval for a testing nerve volley.

ii. It raises the threshold level of e.p.p. for initiating a spike.

In action *i*, the e.p.p. resembles the effect of a cathodal polarization directly applied during a nerve's refractory period (Blair and Erlanger). Actions *i.* and *ii.* are both larger if the e.p.p. is increased by an early second nerve volley or by eserization, and smaller if it is diminished by curarization.

The size of the e.p.p. set up by a testing nerve volley is diminished during the refractory period of the muscle.

We wish to express our thanks to Dr. Bernhard Katz for his valuable suggestions and to the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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ON THE ALLEGED SYNCHRONIZATION OF PROPRIO- CEPTIVE IMPULSES WITHIN SPINAL GANGLIA

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KAYSER has recently compared the spike potentials of proprioceptive sensory fibers in the frog's sciatic nerve and in the 9th dorsal root when the gastrocnemius muscle is stretched. He reports a lower frequency and a higher voltage of the spikes in the dorsal root than in the nerve. These observations led him to the conclusion that "impulses produced by stretching a muscle running separately and irregularly within the nerve, become transformed in the spinal ganglion into synchronized and rhythmic waves of impulses" (Kayser, 1939, p. 504). It seemed worth while to test these conclusions experimentally, since synchronization of impulses is an outstanding problem in the physiology of the nervous system.

RESULTS

Procedure (Part I). If synchronization of impulses actually occurs within the spinal ganglion, impulses starting at slightly different times in two proprioceptive fibers emerging from a single muscle might appear in the dorsal root as fused into a single spike. Therefore in a series of experiments in five bullfrogs we dissected out the sciatic nerve with the 8th and the 9th dorsal roots and with two branches of the medial popliteal nerve from the gastrocnemius muscle. The branches, put on separate electrodes in a moist chamber, were stimulated individually by induction shocks, the interval between which was regulated by a Lucas pendulum opening the primary circuits of two Harvard coils. Dorsal root potentials were recorded on the cathode ray oscillograph when the two nerves were stimulated separately and then together at various intervals in steps of 0.11 msec.

At only *one* setting of the movable key in each experiment did the spike appear truly single. The difference between the lengths of the two nerves (and perhaps a slight difference in conduction velocities) was sufficient to account for the fact that a single spike was seen in the root *not* when the stimuli were synchronous, but when they were separated by a definite small interval. An increment of only ± 0.11 msec. to this interval between the stimuli resulted in a change in the shape of the spike potential in the dorsal root. It therefore appears that synchronization did not take place.

Figure 1 shows the results of such an experiment. *B* and *C* represent records of the spikes in the dorsal root when the proprioceptive fibers in the two nerve branches were stimulated separately. *A* shows four composite spike potentials observed when the two nerves were excited with varying time intervals between the two stimuli. The summit of the composite spike is markedly delayed when the two impulses are separated by an interval of 0.22 msec., at an interval of 0.44 msec. the crests of the spikes begin to separate, and at an interval of 0.55 msec. they are quite clearly separated. The four curves in Fig. 1*D* were calculated from *B* and *C* by adding the ordinates of the curves in *B* and *C* with the peaks coincident and separated by intervals

of 0.22, 0.44 and 0.55 msec. The calculated curves in *D* agree closely with the summated spikes in *A* which were actually registered.

All five experiments agreed in showing that spike potentials reaching the spinal ganglion by way of different fibers do not become synchronized even when separated by intervals of only a few tenths of a millisecond.

Procedure (Part 2). To detect the mistakes that had led to Kayser's statements we repeated his experiments. In one bullfrog and 9 leopard frogs the gastrocnemius muscle was dissected out with the sciatic nerve and the lower end of the spinal column containing the ligated 8th and 9th dorsal roots. The preparation was mounted in a moist chamber with a clamp fixing the distal end of the femur, the nerve and one of the roots were placed on silver electrodes, and different weights were loaded on the muscle by a string passing over a wheel and attached to the tendon.

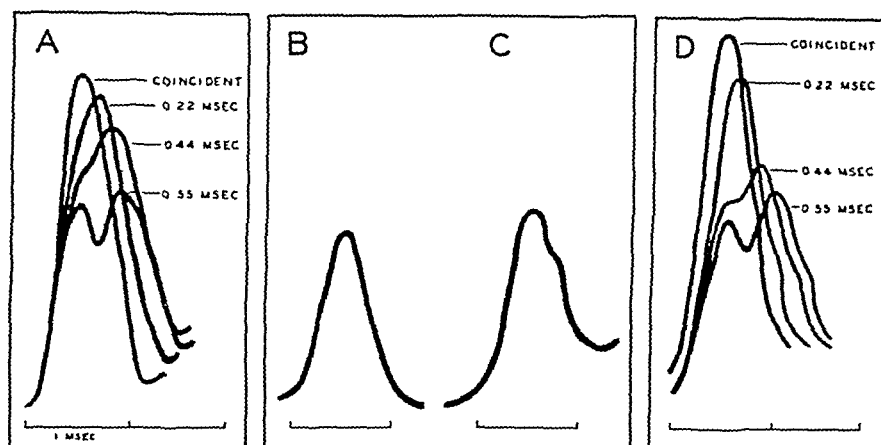


FIG. 1. "Coincident" means coincidence of spike potentials in the dorsal root, not coincidence of stimuli and the intervals represent increments of interval between stimuli, not absolute intervals. *A*, Spikes recorded from the 9th dorsal root when two muscular branches of the medial popliteal nerve were stimulated at intervals as indicated. (The intervals are accurate to within ± 0.05 msec.) *B* and *C*, Spikes recorded in the same way when each branch was stimulated separately. *D*, Calculated summation of spikes *B* and *C* at same intervals as in *A*. (Bullfrog 12/20/40.)

Even when the muscle was loaded with weights of only 10 to 50 g. the proprioceptive impulses recorded from the nerve were so many and occurred at such irregular intervals that it was not possible to identify the potentials coming from single fibers. Typical monophasic recordings of the activity in the nerve immediately upon loading the muscle are given in Fig. 2*A* and 3*B*. Figures 3*C* and 3*D* are from similar experiments, recorded diphasically. As is to be expected, the spikes are much higher in the monophasic recordings than in the diphasic. The highest spikes in these records are probably all chance summations due to transient synchronies.

In monophasic records from the dorsal roots it was often possible to identify the activity of single fibers (Fig. 3*A* and 4*B*). Figure 4*B* shows the potentials recorded from the 8th root of a leopard frog 0.5 sec. after loading the gastrocnemius with 20 g. Besides a few relatively small and irregular re-

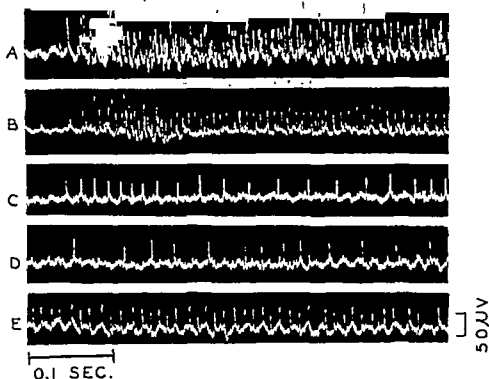


FIG. 2. *A, B, C and D*: Proprioceptive responses recorded monophasically when gastrocnemius was stretched. *A*, from sciatic nerve, weight 50 g.; *B*, from 9th dorsal root, weight 50 g.; *C*, same as in *B* 30 min. later; *D*, from 9th root, weight 10 g. *E*. Spontaneous firing of a single receptor, 9th root (frequency 92 per sec.). Such spontaneous firing was rare in our experiments. (Leopard frog 5/6/41.)

sponses there are two series of spikes occurring at fairly regular intervals at frequencies of 58 and 64 per sec. The first and the last high spikes of this series consist of the two coincident impulses, whereas all those between them

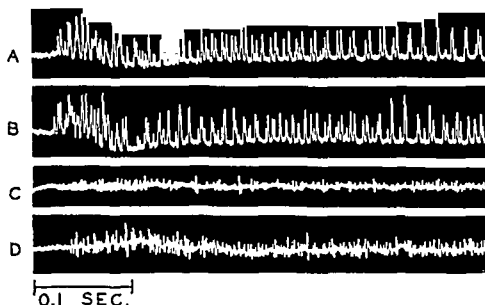


FIG. 3. Activity of proprioceptive fibers elicited by stretching gastrocnemius muscle with 20 g. (3/5/41). *A*, Recorded monophasically from 8th dorsal root. *B*, Recorded monophasically from sciatic nerve. *C* and *D*, Recorded diphasically from sciatic nerve. Amplification in *D* twice that in *A, B* and *C*.

are distinctly separated. Even in the potential before the last, two distinct spikes are clearly distinguishable.

Activity limited to only *one* fiber was occasionally encountered (Fig. 2B, C, D). In Fig. 2C the frequency, initially 68 per sec., dropped to about 30 after a quarter of a second; later it rose again to its initial value. This secondary increase in frequency is similar to the second increase observed by Hartline (1935) in the responses of a single fiber from the eye of *Limulus*.

Generally more frequent and higher summated spikes were found in the nerve than in the root (cf. Fig. 2A and B). In a few cases the potentials in the nerve and in the root looked very much alike (Fig. 3A and B). Since in this frog the 9th root also showed responses very much the same as those represented here, the similarity of A and B certainly cannot be explained by assuming that most of the proprioceptive fibers active in the nerve entered the 8th dorsal root. Furthermore, spikes recorded monophasically were never found to be larger in one of the roots than they were in the sciatic nerve.

DISCUSSION

Synchronization of active neurons has often been encountered in the

central nervous system and to some extent also in peripheral nerves. Adrian (1930) describes synchronization of spontaneously discharging fibers in excised mammalian nerves. "The most reasonable explanation seems to be that an active fiber can cause a slight momentary increase in the stimulus to other fibers and that it can do so owing to the action current which it produces." Such a synchronization may occur if fibers are exposed to a *constant* stimulus, e.g., at the injured ends of the nerve fibers, but Adrian's explanation of course would not hold for a synchronization of fibers each of which responds to a different rhythm imposed on it by its peripheral end-organ. In crab nerve fibers Katz and Schmitt (1939) reported a synchronization and equilization of speed of impulses when an impulse in one fiber slightly precedes that in an adjacent fiber. They explain the phenomenon

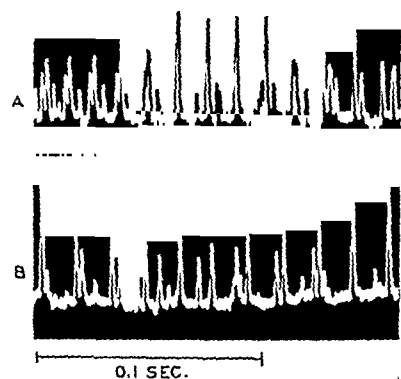


FIG. 4. A, Responses from several fibers, two of which become almost synchronized for about 0.05 sec., recorded from sciatic nerve. B, Two fibers active at slightly different frequencies (64 and 58 per sec.), recorded from 8th dorsal root. (Leopard frog 3/5/41.)

as due to a reduction of the propagation velocity by an interference of the local action potentials.

Kayser inferred a synchronization of impulses within the spinal ganglion mainly because (i) he had recorded larger spikes from the 9th root than from the nerve and (ii) there were fewer spikes in the root per unit time than in the nerve. The difference in size was to be expected, since the potentials in

the root were recorded monophasically (cf. Kayser, 1939, Fig. 1), those in the nerve diphasically.* Also, fewer spikes are to be expected in the 9th root than in the nerve because part of the proprioceptive fibers active in the nerve reach the spinal cord through the 8th and perhaps the 10th roots. Furthermore some of the fibers in the root may sometimes be damaged in preparation or may deteriorate during the experiment.

It is true that transient synchronization may occur from time to time (Fig. 4A), but it is rarely maintained for more than one or two impulses (Fig. 4B). Kayser believed that the large spikes that he recorded from the root were the summations of many impulses, but their fairly constant height, regular rhythm and gradually decreasing frequency are highly characteristic of the responses of a single receptor.

SUMMARY

No synchronization by the spinal ganglion was apparent when two discrete sets of proprioceptive fibers were stimulated with intervals of a few tenths of a millisecond between the two electric stimuli. Experiments in which proprioceptive fibers were stimulated by stretching the gastrocnemius indicated that there was no synchronization because

1. Monophasically recorded spikes were never larger in the dorsal roots than in the sciatic nerve.
2. Typical recordings from the dorsal roots contained two or more simultaneous regular trains of impulses occurring at slightly different frequencies.
3. Responses of equal height occurring at a constant frequency or at intervals that slowly increase with time (adaptation) are characteristic of single-fiber activity. No evidence of a regular continuing sequence of composite spikes was found.

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* The excursions of his spikes below the baseline in his recordings from the roots (cf. Fig. 2 and 5) are due to incomplete damping of the string, as is indicated also by the later oscillations.

HYPERACTIVITY IN MONKEYS FOLLOWING LESIONS OF THE FRONTAL LOBES*

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INTRODUCTION

BILATERAL ablation of the frontal lobes of monkeys is followed by a marked hyperactivity which is characteristic of this lesion. Such animals display a "drivenness" of motor performance reminiscent of the incessant pacing of a caged lion, being characteristically purposeless, repetitive and seemingly interminable.

A spontaneous increase in activity resulting from lesions of the frontal lobes has been reported previously in many animals. In man, bilateral frontal ablation is followed by distractibility, easy excitability and sometimes by overactivity (Brickner 4, Levin 14, Hebb and Penfield 7). Increased irritability is also associated with frontal lobe pathology, as in "behavior problem" children (Levin 14).

In *dogs* and *monkeys* the syndrome of hyperactivity has been described by Ferrier (6) and Bianchi (3); in monkeys by Jacobsen (9); in cats by Langworthy and Richter (12) and Magoun and Ranson (15); and in rats by Lashley (13). It was quantitatively measured by Richter and Hines (16) in monkeys, and by Richter and Hawkes (17) in rats. Increased activity following bilateral removal of area 8 alone (as well as after ablation of the entire frontal association areas, *i.e.* areas 9-12 of Brodmann) has been noted in monkeys (Kennard and Ectors 11), and Richter and Hines (16) believe that the increased activity after frontal ablation is further augmented when the caudate nucleus is injured. Beach (1, 2), however, does not find markedly increased activity after frontal ablations or caudate injury in rats.

Many of the observations on increased activity have been described casually as part of the syndrome of frontal lobe ablation. In man, "drivenness" and hyperactivity have been difficult to analyze, from either the point of view of the cause or nature of the symptom. The papers of Richter and Hines and of Beach are perhaps the only ones dealing primarily with activity changes, and their results are not in accord. Richter and Hines have attempted further systematic analysis. In this laboratory also spontaneous increase in activity peculiar to extirpations of the tips of the frontal lobes was first observed casually (Jacobsen 9; see also Kennard and Ectors 11, Kennard 10), but during the past 5 years more systematic and intensive analysis has been attempted. The results are summarized in the present paper.

* Aided by a grant from the Knight Fund, Yale University School of Medicine.

METHOD

Monkeys have been used, either *Macaca mulatta*, or the sooty mangabey (*Cercocebus torquatus atys*). Activity was measured both before and after operative removal of various portions of the central nervous system by means of kymograph records taken over a two-hour period in which the animal was allowed to move at will in an oblong cage 4 ft. \times 1.25 ft., and 1.25 ft. high. The floor of this cage consisted of a galvanized iron pan which was set upon two pneumatic pads one at each end. These were slightly inflated with air and connected directly with a Marey tambour recording on the kymograph with ink. Thus, movement of the animal resulted in a stroke of the pen and number of strokes equalled roughly the amount of activity. Since the pre- and postoperative differences between amounts of activity were very great, the method was sufficiently accurate (Fig. 1). In many instances a numerical count of strokes was made, but for the remainder records were rated as of low, medium or high normal activity, or of low, medium or high hyperactivity.

Various control conditions were maintained but routine tests were made in the morning with the animals fasting under conditions as quiet as possible. The lights were kept on to insure uniform illumination. Other tests were carried out in a dark and soundproof room, and still others with varying degrees of light. The effect of both prolonged fast and of over-feeding were also tried.

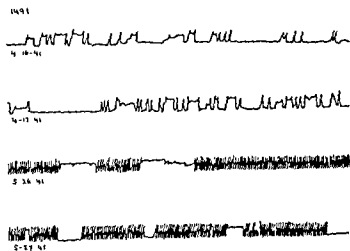


FIG. 1. Changes in activity as result of bilateral ablation of areas 8-12 on April 29, 1940.

DATA

Nature of hyperactivity. A normal monkey, placed in the oblong cage just described, exhibits a variety of behavior depending on its nature and the stimuli of the moment. Thus a two-hour record of such activity may vary moderately, but total activity seen on records made on 3 successive days is fairly uniform. There is considerable variation between individual animals, but in 53 normal animals hyperactivity approximating that in animals after lesions of the frontal areas has been encountered in only 3 instances. Those three monkeys were discarded from the series and no explanation was found for the high level of activity.

Frontal lobe hyperactivity is so typical that the animals may be picked out from a room full of other monkeys by the character of their cage performance. They move constantly back and forth and up and down from the perches. Movement is conspicuously purposeless and repetitive, although its pattern is often too complex to be called perseverative. Such animals, when fed, may be obviously hungry yet moving so fast that they cannot eat. They will pick up bits of food, jump upon the perch, take a bite, put the food down, grab it again and repeat the performance indefinitely. Their easy distractibility is noticeable at all times. Stimuli evoke a greater response than in the normal animal, and emotional stress always increases activity. Otherwise, their personalities remain unchanged, they are neither more irritable nor more pleasant, more curious nor more fearful than before operation. A cer-

tain lack of discrimination seems also to be present. Any object may be put into the mouth, and the animal may attempt many times to reach something well outside the bars of the cage; the purposelessness of the hyperactivity thus indicates lack of appreciation of environment.

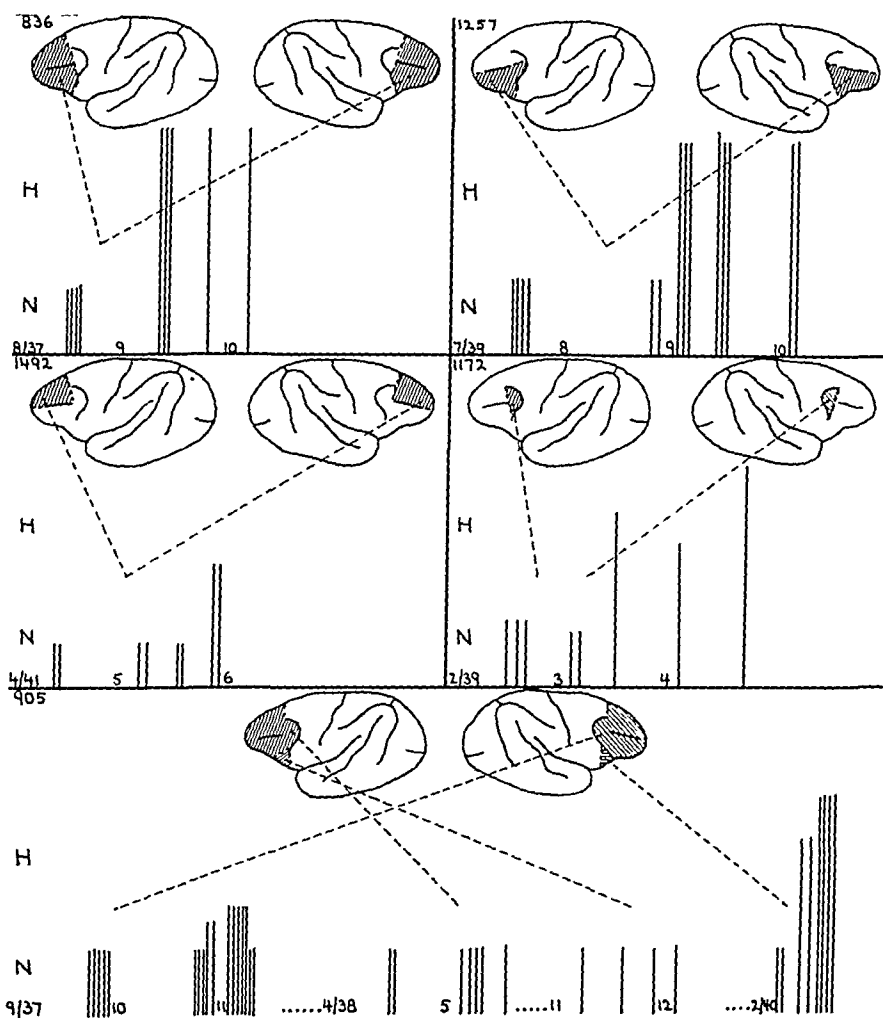


FIG. 2. Effect on activity of various bilateral lesions of frontal lobes. N = normal and H = hyperactive range of activity. Time in months indicated by figures on baselines. Time of operation shown by dotted lines connecting sketch with activity record.

The hyperactivity is usually preceded by a period of hypoactivity immediately following the operation which may last a few days or weeks. During this time, the animals appear confused, lethargic, slow and difficult to rouse. They often have to be fed by force since they show no interest in food or other objects. This is not a manifestation of the so-called postoperative

shock, for it is specific to lesions of the frontal lobes and does not occur following bilateral lesions elsewhere. Also, in many instances it lasts much longer than does the immediate effect of the operation.

2. *Localization within cerebral cortex.* Hyperactivity of this sort appears only after ablations from the frontal lobes. It occurred most rapidly and in greatest degree in 12 monkeys which had bilateral ablation of areas 8-12 (Fig. 2). Bilateral ablations of areas 9-12 also produced particularly marked hyperactivity in five animals. Removal of area 8 from both sides produced hyperactivity in 6 cases. For some days these monkeys all showed other symptoms of removal of area 8, i.e., limited conjugate deviation of the eyes, a mask-like face and fixed gaze together with a marked slowness in all movement. But after removal of area 8 alone the increase in activity was never as great as that which followed removal of the larger areas; and, when at a second operation, areas 9-12 were added to the ablations of area 8, total activity again increased.

Removal of area 9 bilaterally in 3 cases was followed by a slight increase in one instance, but no increase in the other 2 cases (Fig. 2). In contrast, removal of the lateral portions of the frontal association areas, areas 8, 10, 11 and 12 without area 9 in 4 cases had the effect of increasing activity much more markedly. The subsequent removal of the remaining portions of the frontal lobes increased activity again in both cases.

Involvement of the caudate head or of the putamen seemed in our cases to have no effect on the amount of activity. It was undamaged in the majority of cases after bilateral removal of areas 9-12 in which hyperactivity was extreme, although it was injured in some instances. Moreover, when the caudate head alone was removed bilaterally via a mid-line approach and section of the corpus callosum, there was no increased activity.

In our experience, *unilateral* ablation of any of these cortical areas has had slight effect on activity. In only 3 cases out of 11 in which activity has been measured before and after unilateral ablation of frontal association areas has hyperactivity appeared, and in these 3 cases it was slight, and it increased greatly when the opposite area was later amputated.

Effect of other cortical lesions on hyperactivity induced by lesions of frontal association areas. Bilateral extirpations from parietal or temporal lobes do not produce hyperactivity. Bilateral removal of area 6 is followed by transient hypomotility which is most marked when the lesions have been simultaneous on the two sides. With recovery from this, however, there is no hyperactivity. Moreover, no lesion or combination of lesions made subsequently in other parts of the cortex will diminish the hyperactivity which has appeared after lesions of frontal association areas, unless such profound paresis develops that the movement of the animal is interfered with, or unless vision is sufficiently restricted, by occipital lobectomy.

In one monkey when all of one hemisphere had been taken out, together with areas 8-12 on the other side, hyperactivity occurred. In another, from one hemisphere areas 9-12; areas 6, 4, 3, 1, and 2; and areas 17 and 18 were

removed in three successive operations in that order. No hyperactivity resulted but at a 4th operation in which the remaining areas 9-12 were extirpated, hyperactivity became marked. A third monkey had the left frontal lobe and area 8 of the opposite side extirpated. Hyperactivity followed. Then area 6 was removed without altering activity although temporary partial paresis occurred. Finally, when the remaining area 4 was extirpated marked paresis appeared and hyperactivity consequently became impossible.

Factors affecting hyperactivity. (a) *Sound.* Since the animals were noticeably distractible the effect of removal of distraction was tried. The activity cage was placed in a sound proof room. Records there showed no diminution in activity, but rather a slight increase, for greater regularity of movement was present obviously due to the fact that there were none of the momentary pauses which usually appeared because of distraction by some slight sound. In one monkey, activity was normal after section of both eighth nerves. Subsequent ablation of both frontal association areas then produced hyperactivity.

(b) *Light.* Whenever a hyperactive monkey was placed in the dark, all activity ceased. This occurs also in normal monkeys and might be expected since their tree-dwelling habits require light for safety in movement. It seems more remarkable, however, in the hyperactive animal. Two monkeys from which the eyes had been enucleated had all of areas 8-12 removed, one before and the other after enucleation. No hyperactivity resulted. A third animal was tested from which both occipital lobes had been removed in infancy and which was at the time of testing, nearly three years old. The pupils of this macaque responded definitely to light as is to be expected from the work of Marquis and Hilgard (16). In addition it would move its head and eyes to follow strong light, but no object vision further could be demonstrated. Its activity prior to ablation of the frontal areas was normal. Following their removal it became slightly more active, although true hyperactivity never appeared. A fourth animal was made hyperactive, and then had both occipital lobes removed. Hyperactivity ceased.

The effect of continued light was, as would be expected, the opposite of dark. Activity measured during the night if the lights were kept on remained high. One animal kept in the light for 48 hours continued to be hyperactive for this interval, although at the end of the period there were brief pauses during which it would lie down or sit, apparently because of fatigue, only to jump up again and resume pacing.

(c) *Hunger.* Since the frontal lobes are suspected of having direct effect on autonomic functions (8, 18, 19), increased metabolism or possibly increased hunger "drive" was a possible source of activity. Therefore monkeys were tested during fasting and overfed conditions. It was found that normal controls fed a fixed diet at a fixed time daily were slightly more active before feeding than during the hour immediately after. The same effect appeared in the hyperactive monkeys, but it could not be seen that hunger affected activity more in these than in the normal animals.

Metabolism. Since basal rates in monkeys are somewhat difficult to obtain (Bruhn 5), a series of animals was next placed on a weighed diet, before and after ablation of frontal association areas. Weight and intake were compared (Fig. 3). Again, no marked signs of increased metabolism were found

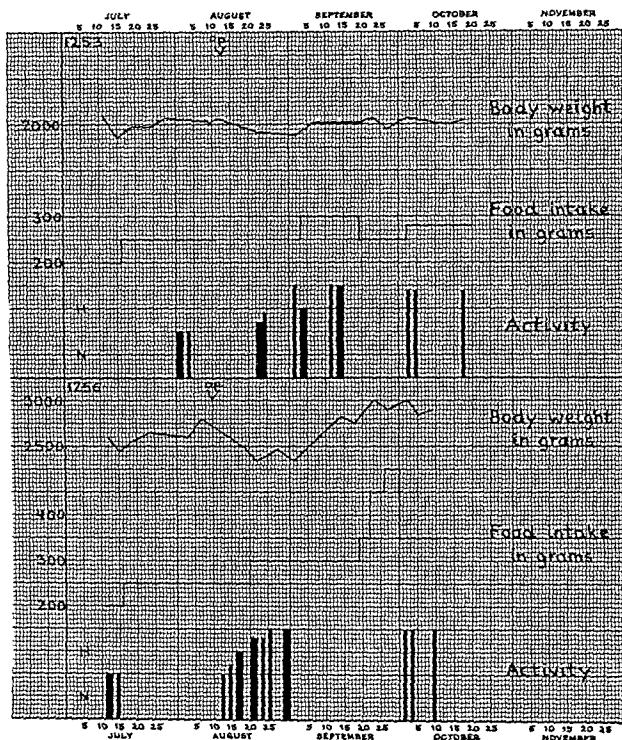


FIG. 3. Records of two *Macaca mulatta*, made hyperactive by removal of areas 8-12 during period of controlled feeding, no loss of body weight, although activity increased. Arrow marks time of operation. N=normal, and H=hyperactive range of activity.

following operation. The hyperactive animals gained weight slightly more slowly than the normal or required slightly more calories for equal weight gain with the normal, but no more than might be necessary for the increased work of increased activity, and far less than was necessary for hyperthyroid animals.

Five monkeys have been placed on daily thyroid dosage sufficient to increase their metabolic rate to marked degree. These were made hyperactive before or after thyroid dosage. The results of these investigations will be reported in detail at a later date when fully completed. Figure 4 shows the marked differences between hyperthyroid and hyperactive animals, which had totally opposite responses in almost every respect. On a weighed fixed diet hyperactive animals lost little weight, although activity increased enormously.

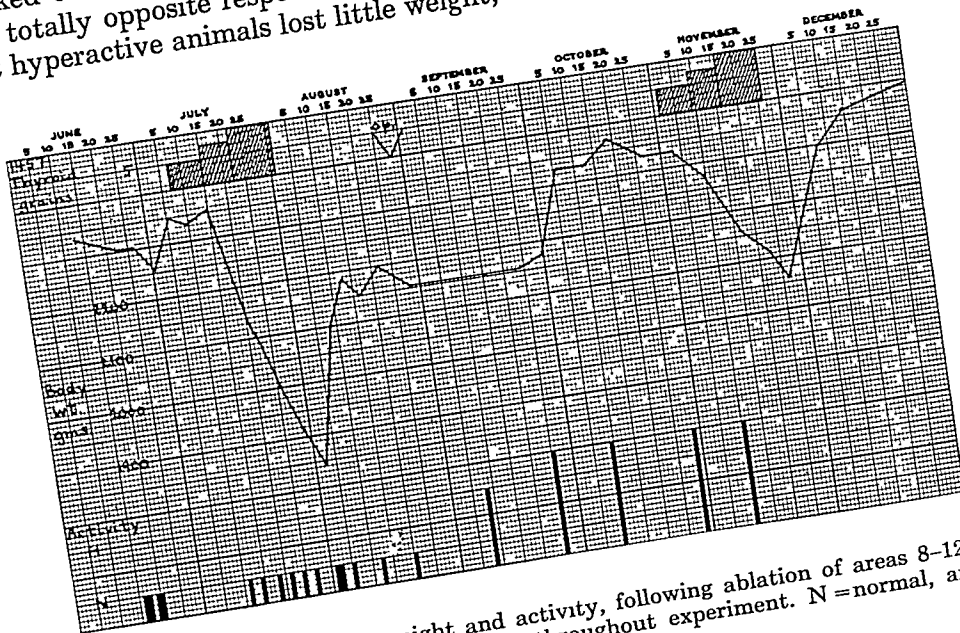


FIG. 4. Changes in body weight and activity, following ablation of areas 8-12 and during thyroid medication. Standard diet throughout experiment. N=normal, and H=hyperactive range of activity.

mously. Those with increased metabolism, on the other hand, lost weight rapidly, but increased activity but little (Fig. 6). Pulse and blood pressure rose significantly in hyperthyroidism but little if at all in hyperactivity. The gastrointestinal peristalsis was measured by means of carmine tablets which were given by mouth, the time being recorded when color appeared in the feces. Figure 5 indicates that the rate of peristalsis did not decrease during hyperactivity, but that it diminished from the normal of 18-24 hours to 2, 4, or 6 hours in the hyperthyroid monkeys. It seems certain therefore that neither increased metabolism nor changes in the autonomic functions of the gastrointestinal tract can be responsible for hyperactivity.

In the three species of monkeys operated upon (macaque, mangabey and cebus) all of which walk as quadrupeds, the pacing repetitive motion has been extreme. In the chimpanzee, which walks for the most part upright, and has a more complicated frontal lobe, there is a somewhat different response to bilateral frontal lobe ablations. Two such chimpanzees have been prepared in the laboratory. Both showed restlessness and distractibility of sufficient

intensity to be noted independently by several observers. There was, however, little of the pacing restlessness of the quadrupedal primates.

DISCUSSION

The surprising phenomenon of an enormous increase in activity appearing after injury to the frontal lobes in animals whose behavior is otherwise normal has been redemonstrated in this series of monkeys. The purposeless and repetitive elements of the hyperactivity, together with its "driven"

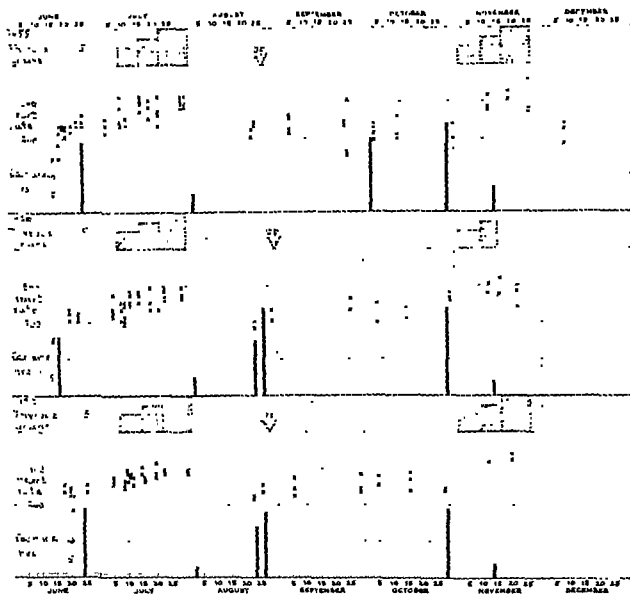


FIG. 5. Effect of thyroid on heart rate and intestinal peristalsis (passage of carmine through G. I. tract) before and after bilateral ablation of areas 8-12 in 3 *Macaca mulatta*.

character have been conspicuous as noted by all previous observers, but activity is so general an attribute that analysis of the factors effective in its production is difficult.

Our data, however, indicated that these alterations cannot be ascribed to changes in metabolism or to other autonomic disturbances, for no autonomic function was found affected. Furthermore a normal or a hyperactive monkey, when given thyroid, exhibits increase in blood pressure, heart rate and in-

testinal peristalsis, together with all the characteristics shown by hyperthyroidism in man, *i.e.*, loss of weight, increased appetite, nervousness, sweating and easy excitability, but activity will increase only slightly during hyperthyroidism.

Attempts to localize within the frontal association areas one region which

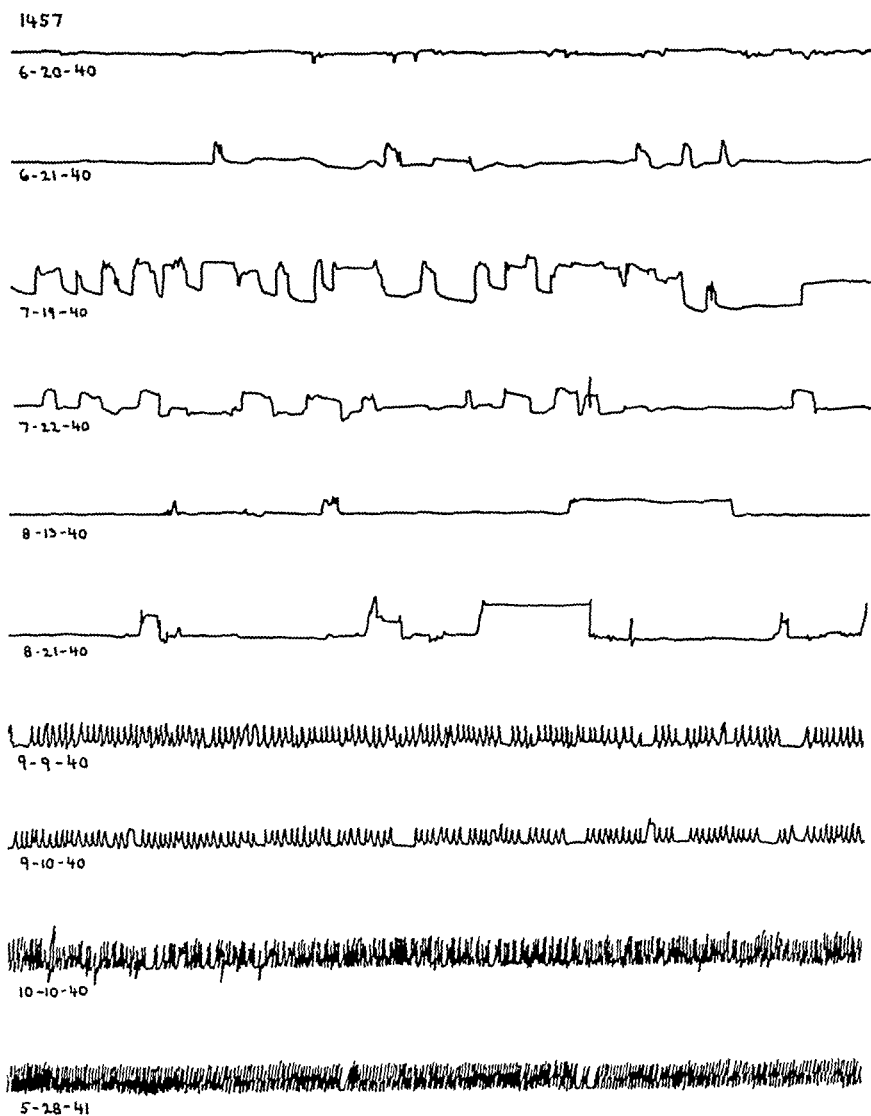


FIG. 6. Changes in activity as result of thyroid medication and bilateral ablation of areas 8-12 in a *Macaca mulatta*. Thyroid from July 7-31. Bilateral ablation of areas 8-12,—Aug. 29, 1940.

is responsible for hyperactivity have failed. Bilateral removal of the total area, or of all of it except area 8, undoubtedly causes greater activity and more quickly than do partial lesions. However, Richter and Hines have

found moderate increase in activity after removal of these areas from one side only; bilateral extirpation of area 8 alone causes marked hyperactivity, and ablations of fractions of areas 9-12 likewise increase activity moderately. If, however, one part is removed and at a later date the remainder extirpated, increased activity occurs after the second operation as well as after the first. The entire region seems thus to affect activity since removal of no part causes as great a change as does removal of the whole.

The lateral portions have more effect than do the mesial. The record of one animal is of interest in this connection. It can be seen from the chart (Fig. 2) that activity changed little in this instance after the first three operations, but that following a fourth which removed a final bit of the lateral tissue, a great increase in activity occurred.

What then can be the mechanism responsible for a change in total activity of an organism without other change in motor, sensory, or autonomic function, a change roughly proportional to the amount of tissue removed? The theory of "release of function" is inviting for it is used in relation to other functions of the frontal lobes such as postural regulation, for removal of specific portions of frontal lobes results in exaggeration of function; integration of the function in question can therefore be attributed to this area under normal conditions. These release mechanisms have another factor in common, which is the variability in time at which release becomes evident after the lesion has been made. Hyperactivity, hyperreflexia and spasticity appear at an unpredictable time following operation and there is nothing in the data which explains the time of appearance. Neither species nor age, as far as can be seen, affect hyperactivity, nor does the length of time under anesthetic, or the severity of the operation. Yet the hyperactivity may appear on the day after operation, or it may not be seen in full intensity until a month has passed. The intensely hyperactive animals do, however, show changes earlier than do those which are to become only moderately hyperactive. The same relationship to time appears in the "release phenomena" of reflex grasping or spasticity. There is as yet no valid theory to account for the temporal progression of this reorganization.

There remains one fact which may contribute to the causes of hyperactivity, namely that it is altered by visual stimuli, although auditory stimuli have no such obvious effect. When this work was in its early stages it was found that ablation of area 8 from one hemisphere produced inability to recognize objects in the contralateral field of vision, and that bilateral ablation of area 8 was followed by a temporary condition in which the monkey appeared dazed and showed no evidence of recognizing objects visually (10). It was thought that this might be a cause of hyperactivity and distractability. For, if objects were not recognized they might be ignored. There is still possibility that this, or a like mechanism affects activity, but it cannot be the whole cause since the failure to recognize objects is a transient affair, whereas hyperactivity is permanent and occurs when areas 9-12 have been removed without visual symptoms, if area 8 is undamaged. That areas 9-12 affect

vision to some extent is evident since although their removal causes neither conjugate deviation of the eyes nor failure to recognize objects seen, their ablation simultaneously with area 8 always results in increase in intensity and duration of these symptoms (11).

Jacobsen has shown (9) after bilateral ablation of the frontal association areas, that monkeys and chimpanzees lose their "immediate memory." They are unable to pick the correct inverted cup under which food has been placed in their sight if the field has been cut off from their vision for a short time afterward, although previous to operation they can make correct choice under such conditions. This loss of "delayed response" may be associated with the effect of light on activity as indicated by the subsequent work of Malmo (unpublished) who, substantiating Jacobsen's findings, has shown that if light is greatly cut down the monkey retains some ability to recall although it is limited when compared to preoperative performance. Both the hyperactive condition and the test situation of "immediate memory" are thus affected by visual stimuli, and distractability becomes directly proportional to light in both instances.

There are no data on the effect of visual stimuli on man without frontal association areas. But restlessness and distractability are recorded by many observers and discussed recently by Brickner (4). The compulsive behavior of the "behavior problem" may have a like background producing restlessness (Levin, 15).

The chimpanzee, now a familiar intermediary between man and monkey in the elucidation of problems of the central nervous system, has here proved a connecting link also. Man, monkey and the chimpanzee it is agreed, all show an exaggeration of the compulsive type of behavior when the frontal lobes are damaged. In all three, restlessness and distractability are increased. The monkey then walks on and on incessantly, but chimpanzee and man have more complex compulsive patterns. Increased activity can be found, but over-reaction to the stimuli inducing emotion and to those which require a knowledge of previous conditions are most conspicuously affected (Brickner, 4).

Experiments with the monkey and chimpanzee give information on another manifestation common in frontal lesions in man, namely the lethargy, or hypoactivity which is found with some frontal lobe lesions. Dullness, slowness, inertia or hypomotility are as often recorded as are restlessness or overactivity in these cases, and many times this cannot be due to generalized increased intracranial pressure.

In the monkey and chimpanzee hypomotility follows bilateral ablation of area 6 or temporarily of area 8. When the rostral portion of area 6 is removed paresis is slight but reflex grasping marked. Confusion and unresponsiveness to all types of stimuli are present, and perseveration is evident. In the monkey this will disappear in the course of a few weeks; in the chimpanzee it becomes less marked but lasts indefinitely. Transient symptoms of bilateral removal of area 8 are also confusion and hypomotility combined

with, and no doubt due to, a failure to recognize objects seen (10); a fixity of eyes and of facial expression adds to the impression of dull and unresponsive apathy.

It is therefore possible that in man also, hypomotility may be a manifestation of bilateral involvement of the frontal lobes in the regions of areas 6 and 8, whereas the restless driven patient may have destruction of more rostral regions which are relatively silent fields for physiological investigation, but which are more directly concerned with psychological properties.

SUMMARY

1. In monkeys and chimpanzees ablations from the frontal association areas (areas 8-12 of Brodmann) result in increased total activity which is beyond that ever seen in the normal animal.

2. This hyperactivity is characterized by purposelessness and by repetitive continuation. It is permanent.

3. Partial ablations, if bilateral, produce some increase in activity (bilateral area 8, bilateral area 9, or bilateral areas 10-12), but none of these cause as great increase as does removal of all of the frontal association areas.

4. The increased activity has not been found to be related to increased hunger or metabolism; or to changes in the autonomic system.

5. Hyperactivity is markedly affected by visual stimuli. It disappears in the dark, or when the animals have been deprived of vision either by enucleation of the eyes or occipital lobectomy. Absence of auditory stimuli has not the same effect.

5. Hypomotility in monkeys and chimpanzees is related to lesions of the rostral portions of areas 6 and to area 8.

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THE SPINAL MECHANISM OF THE PYRAMIDAL SYSTEM IN CATS*

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IT IS THE purpose of this study to examine the functional organization of that portion of the spinal integrating mechanism which is subject to activation by the pyramidal system.† To this end it is imperative that other sites of integration be eliminated, and that pyramidal impulses alone be allowed to enter the spinal cord. Stimulation of the "motor" cortex may not be employed, because cortical facilitation will result (Graham Brown, 1915a; Adrian, 1936), and because of the inevitable participation of the extra-pyramidal system in the distribution of activity to the spinal cord (Schäfer, 1910; Tower, 1936). Just as complete interruption of the pyramidal tracts without damage to other systems may only be accomplished by section of the pyramids in the medulla (Marshall, 1936), so only at the pyramids may the tract be stimulated without activation of other systems, and then only by the use of additional precautions. Other elements within the medulla, notably the reticular formation, may be activated by pyramidal collaterals or by direct extension of the stimulating current. The latter mode of activation has been known to occur even when electrodes designed to concentrate the stimulating current across the pyramids have been used. It has been necessary, therefore, to interrupt all pathways other than the pyramids at a level caudal to that of the stimulating electrodes. In consequence the preparations are essentially spinal, for conversion from the decerebrate state to the spinal state, in the cat, accompanies lesions of the vestibular nucleus or its direct pathway (Fulton, Liddell, and Rioch, 1930a, b).

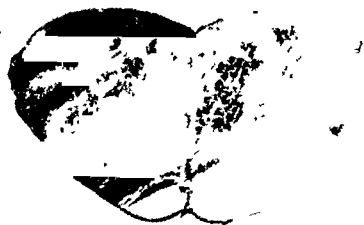
It is not enough that pathways other than the pyramidal tract be interrupted below the site of the stimulating electrodes. A complete transection cranially is necessary to prevent ascending activity from reaching the cortex by various channels (antidromically along the pyramidal fibers, thence by collaterals, or dromically through the medial lemniscus and thalamus, etc.), there to activate pyramidal neurons. With all the foregoing precautions, the necessity for which has been shown by experience, the activity reaching the spinal cord as a result of a single pyramidal stimulus is a controlled single pyramidal volley.

Cats have been used throughout the present investigation. These were lightly narcotized with Dial or nembutal. Supplemental ether was regularly

* A preliminary account of some of the present results was presented at the meeting in Chicago of the American Physiological Society (Lloyd, 1941c).

† The definition of the pyramidal tract used herein follows from the descriptions of Türck (1851, 1853) and Flechsig (1876), i.e. the pyramidal tract consists of those fibers which pass through the pyramid to the spinal cord. For a discussion of the use of the term pyramidal tract cf. Marshall (1936).

used until the preparation was decerebrated or rendered essentially spinal. Most of the preparations made in this way were at the upper limit of irritability beyond which the present type of experimentation is not practical, even with the spinal cord held in position by a system of spinal clamps. The slightest movement of the nervous tissue under observation is inimical to the successful recording of "spike-like" activity with rigid microelectrodes. To describe further the functional state of these preparations, it may be noted



that they usually exhibited a strong tonic motoneuron discharge (Fig. 12A), which in the absence of vestibular background was probably maintained by the constant influx of afferent impulses from the periphery (Barron and Matthews, 1935). A record of this tonic afferent activity will be presented in another connection (Lloyd, to be published). It is probable, in the light of recent studies (Marshall, Woolsey, and Bard, 1941; Lloyd, 1941a) that the low concentration of barbiturate present in these preparations (some were observed as long as 14 hours after the administration of nembutal in amounts sufficient for initial basal narcosis) did not interfere appreciably with primary responses to pyramidal stimulation.

Stimulating electrodes were placed through the floor of the fourth ventricle until the bared tips embraced

the pyramid at the level of the trapezoid body. Cranially a complete transection was placed at the collicular level. The lesion caudal to the electrodes was accomplished by means of a spring guillotine. As the blade of this guillotine passes ventrally through the medulla until the curved free border reaches the basiocciput, a central notch in the blade permits the pyramids and basilar artery to escape damage. Figure 1 illustrates, in a macroscopic preparation, the extent of the standard lesion produced by the guillotine at the level of the inferior olives. The medulla was removed from each preparation at the end of the experiment, and the lesion inspected after fixation.

Records were obtained by means of fine steel wire microelectrodes insulated but for the tip. These could be placed at will in the spinal cord with the aid of a micromanipulator, used with due regard for deformation error. Approach to regions of the gray substance from various angles in many experiments has diminished the hazard inherent in mechanical error. The highest degree of accuracy cannot be claimed in the absence of histological control;

however, the latitude in localization which has been allowed for in the responses to be described is ample to compensate for the errors involved.

Conduction in the pyramidal tract. A single shock to the pyramid results in a volley of impulses which is conducted caudally throughout the length of the spinal cord. The response of the pyramidal fibers may be recorded from a highly localized area equal to approximately 1 sq. mm. of the cross-sectional area of the cord, and situated as expected in the cat, in the lateral column adjacent to the dorsal horn (cf. Spitzka, 1886; Lenhossék, 1889; Bechterew, 1890). Figure 2 illustrates the pyramidal tract response to single shocks recorded at various conduction distances. One would expect the pyramidal fibers conducting impulses past the recording microelectrode to yield impulses recordable as triphasic spike potentials (for a recent discussion cf. Lorente de N6, 1939, p. 430 *et seq.*). An attempt to obtain such records even at short conduction distances has been only partially successful. For example, Fig. 2A shows a small negative deflection recorded at a conduction distance of 6.8 cm. If the recording microelectrode is thrust boldly into the region occupied by the pyramidal fibers, or having passed through that region is brought back along its own track, a large, predominantly positive potential results. Figure 2B which shows such a positive potential, was recorded from the same point as was Fig. 2A. Similar effects have been noted by Therman (1941) when attempting to record from the medial lemniscus, and by Bishop and O'Leary (1941) in the superior colliculus. The origin of the predominantly positive responses, such as are shown in Fig. 2, is not entirely clear. Part at least of the initial positive deflection may be ascribed to the impulses approaching from a distance. Consequently it is difficult to designate exactly the time of arrival of impulses at the recording electrode from such records. Damage is an undoubted factor, but some of the recorded impulses reach or pass the electrode, for there are spike potentials in the records having a prominent negative phase, although they are submerged by positivity. The resultant records probably indicate a dispersed discharge



FIG. 2. Activity instituted by single pyramidal volleys and recorded by microelectrode from the pyramidal tract at the conduction distances designated in centimeters at the right. A to D from one preparation. E from another preparation for comparison. Time in msec. Further description in text.

in a number of elements, some of which are blocked at a short distance from the electrode, some of which are blocked at the electrode, and some of which are unblocked.

Records 2C, D, and E were obtained in the same way as was 2B, but at the designated conduction distances. Records 2A to D were obtained from a single preparation. Record 2E is from another preparation for the purpose of comparison. A slope of the latencies in 2B to D reveals a conduction rate of approximately 63.5 M per sec. for the most rapidly conducting fibers of the tract. This value was approximated in all the experiments of this series.

Determination of the lower limit of pyramidal fiber velocities is fraught with uncertainty. The activity as recorded from the tract has no clearly defined termination. The tract itself has intimate anatomical relationship with cellular elements which are known to discharge in response to the tract impulses (cf. next section). Thus, although the pyramidal response certainly suffers considerable temporal dispersion, no definitive value may be given for the lower limit of velocities. A study of the records suggests that an estimate of 18 M per sec. is not too low for the lower limit of velocities, although in view of the large numbers of very fine fibers to be found in the pyramidal tract of the cat (McKibben, and Wheelis, 1932; Lassek and Rasmussen, 1940), this value may be far too high.

The activity illustrated in Fig. 2 is the only tract activity that has been recorded from the white columns of the spinal cord following pyramidal stimulation, as would be expected from a consideration of the anatomical studies of Lenhossék (1889) and Bechterew (1890) on the cat's spinal cord.

It is important to recognize the implications of the fact that the pyramidal tract, subsequent to synchronous activation at the level of the medulla, delivers into the lumbar enlargement, by virtue of dispersion, a prolonged asynchronous discharge. In consequence some features of the tract activity will resemble those of a diffuse nuclear discharge. The major principle is: that time lost by slow conduction is in some measure the formal equivalent of time lost in the synaptic relaying of otherwise rapidly conducting pathways. For example, it will be seen that the most rapidly conducted impulses evoked by a second pyramidal shock will be capable of summation with the more slowly conducted impulses evoked by an antecedent similar shock, given an interval of several milliseconds between shocks and a similar distribution of terminal knobs.

RESPONSES OF THE GRAY SUBSTANCE

(1) *The external basilar region (Cajal).* Following a single shock to the pyramid, and the consequent arrival of a dispersed volley of pyramidal impulses in the lumbar enlargement, certain interneurons within the cord become active. The resulting activity is discernible often only as a slow potential change of some 40 to 45 msec. duration. Under favorable conditions however, a burst of small spike potentials occupying the period of the slow potential wave is recorded. An experiment of this kind is presented in Fig. 3. Record 3A shows the level of noise and activity maintained in the absence

of any specific stimulation. The recording tip of the microelectrode was placed in the extreme lateral margin of the base of the dorsal horn (external basilar region, Cajal, 1909). The anatomical position is confirmed by the appearance in the record (3B) obtained with a single pyramidal volley, of both pyramidal impulses (p) and nuclear discharges (e). The pyramidal impulses recorded in Fig. 3 are probably occupying pyramidal collaterals to the gray substance. The pyramidal impulses begin after a latency of approximately 4 msec., are again of positive sign (indicating damaged bundles of fibers), and are accompanied almost immediately by a flame of small spike potentials, the latter making up a discharge not greater than $50\mu\text{V}$. in amplitude with a duration of some 40 msec. It seems certain, by reason of the fine and obviously non-unitary character of the nuclear discharge, that it may be assigned to a dense population of small elements situated at the lateral aspect of the base of the dorsal horn, and intermingled with fascicles of pyramidal collaterals.

The close functional relationship between the pyramidal tract and nuclear elements at the base of the dorsal horn forces the opinion that the pyramidal fibers impinge, either terminally or collaterally, upon these elements. This interpretation agrees best with the view held by Schäfer (1899), who stated that the pyramidal fibers enter the gray substance at the base of the dorsal horn and end in relation to cells of that region. Other zones of termination are not excluded; for instance, although the type of discharge at present under consideration appears to be more prominent at the external basilar region of Cajal, it is probable that small elements reacting to pyramidal stimulation in a similar manner are more widespread. A suggestion of low amplitude discharges is to be found in other regions following single shocks to the pyramids (Fig. 5B, 6A and G). It is difficult to affirm that these discharges in other regions result clearly, from the pyramidal stimulation. Hence it would be gratuitous to assume, on this basis alone, a more widespread distribution of pyramidal fibers, although

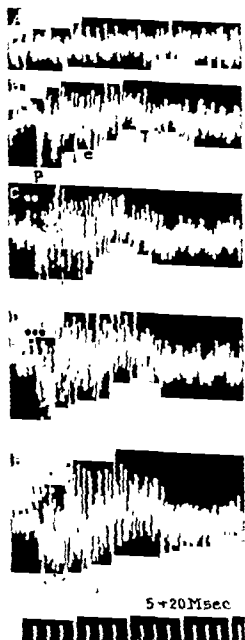


FIG. 3. Activity recorded at the extreme lateral aspect of the base of the dorsal horn. The stimulation artifacts of the pyramidal shocks are marked by dots. Pyramidal impulses and nuclear discharges are identified in B (p and e respectively). Time, in 5 and 20 msec. intervals, is shown at the bottom. In all figures where there are two time designations, these are for the small and large divisions respectively. Further description in text.

some histological studies on the cat do point to this direction (Hoff, 1932).

Records 3C, D, and E show the manner in which the fine basilar discharge is intensified by the addition of successively greater numbers of pyramidal shocks. It is only after a degree of intensification approximating that seen in Fig. 3D and E, that other types of activity have been found in other regions of the gray substance, *i.e.* intensification of activity in one region is accompanied or followed by spread of activity to other regions. The active regions, subsequent to spread, include the solitary cell region of the dorsal horn and the intermediate region.

(2) *Solitary cells (Lenhossék) of the dorsal horn.* The responses obtained in this region have all the characteristics that would be expected from a group of large cells sparsely scattered through a "matrix" of smaller elements. The correlation between functional picture and histological description (Lenhossék, 1895; Cajal, 1909; Bok, 1928, etc.) appears to justify the argument that the discharges under consideration are recorded from the solitary cells of the dorsal horn. The responses are localized, and when found, are relatively uniform in amplitude and fairly regular in frequency when subjected to a prolonged pyramidal tetanus (Fig. 4). At no time in this series of experiments have two similar units of the solitary cell type been detected at the same microelectrode position. On the basis of general experience this fact would indicate that the units are few in number and widely spaced. In general, all the criteria upon which the assumption of unitary response is customarily based (*cf.* Adrian and Bronk, 1928, etc.) are satisfied. The amplitude of the spike potentials recorded from these units is large, being usually of the order of 2mV. It may be stated parenthetically that the only larger spike potentials (up to 6mV) which have been encountered in the spinal gray substance so far are those attributable to motoneurons.

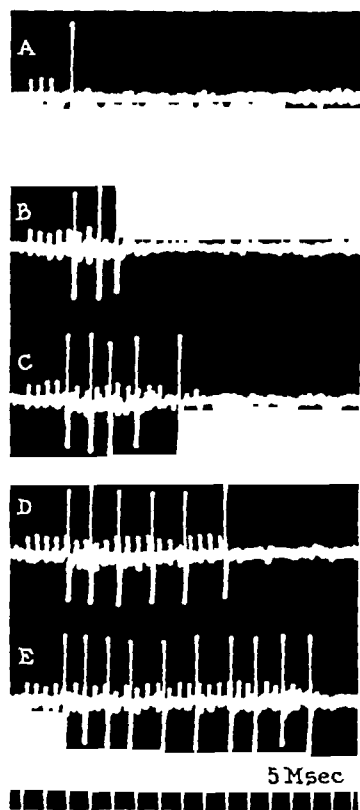


FIG. 4. Activity found in the center and base of the dorsal horn. The small regular deflections are the recorded stimulus artifacts of the pyramidal shocks. The large spike potentials (about 2 mV.) constitute the solitary cell response to pyramidal stimulation.

Figure 4 illustrates the characteristic discharge of a solitary cell unit of the dorsal horn when driven by pyramidal tetani of increasing durations. As seen in Fig. 4A, three pyramidal shocks are necessary to produce the first and single response of the unit. As the tetanus is lengthened (4B to 4E) subsequent discharges occur. A glance at the records of Fig. 4 suffices to show that

there is a roughly direct relationship between the duration of the pyramidal tetanus and the number of discharges obtained from this unit. Even so the discharge frequency bears no direct and exact relationship to the stimulus frequency.

The solitary cell discharge takes place on a background of small cell activity that may be identified in the observations of Fig. 4. It is probable that the highly asynchronous activity of the small units in this region, as well as in the external basilar region, constitutes a slowly changing, statistically smooth over-all excitation to the large solitary cell units, and that these latter in turn respond at intervals determined in part by their own properties (cf. Lorente de N6, 1938, p. 226). The facts, (i) that the solitary cell units discharge only after a 9 or 10 msec. total latent period, (ii) that summation of influences from several pyramidal shocks is necessary to procure a discharge, and (iii) that the stimulus frequency is not evident in the response (cf. with Fig. 7) suggest that the inter-nuncial contribution to the solitary cells is of relatively greater importance than is that of the pyramidal fibers themselves. It is not possible to exclude pyramidal fibers from synaptic relationship with the solitary cells. Whether or not the solitary cells represent important relays distributing pyramidal activity of the motoneurons of the same side depends naturally upon the distribution of the solitary cell axons. Some of these cells have axons passing through the ventral commissure to the opposite side, some have uncrossed axons reaching to the lateral columns (Cajal, 1909; Bok, 1928). The latter might be regarded as reaching the motoneurons of the same side. It is furthermore possible, on the basis of time relationships, that the solitary cells relay activity to the intermediate region.

(3) *The intermediate region, including the intermediate gray nucleus of Cajal.* Units throughout the intermediate region discharge impulses in response to a short train of shocks to the pyramid. The spike potentials yielded by these units have an amplitude of $200\mu\text{V}$. to 1 mV, in general, intermediate in amplitude between the spike potentials of the two groups already considered. The individual units of the intermediate region are not as discrete as are the solitary cells, as evidenced by the fact that

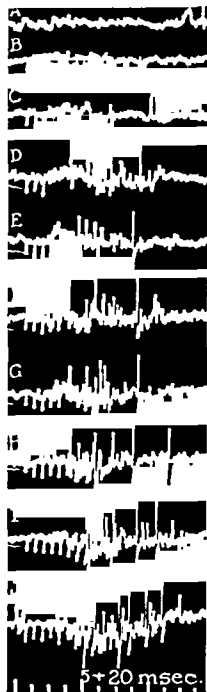


FIG. 5. Activity in the intermediate region resulting from one (B) to nine (J) pyramidal shocks. The level of "resting" activity is shown in A. Further details in text.

even the most favorable records usually contain spike potentials from several similar elements. The intermediate response is most prominent in the ventral part of the intermediate region.

The latent period for activity of the intermediate type varies usually between 12 and 20 msec. in duration. The method of direct recording from a few units is not the most satisfactory method for observing the average discharge latency of a neuron pool, since quite wide variations often occur between successive observations. The values given are thus of necessity estimates

based upon a large number of observations in a number of experiments. The frequency of pyramidal stimulation is a factor influencing the latency of response in the intermediate region, as elsewhere. Various examples to illustrate the latency for discharge in the intermediate region may be found in Fig. 5 and 6.

Figure 5 demonstrates the effect of applying successively longer trains of pyramidal shocks upon the activity in the intermediate region. The microelectrode in this experiment was placed 2.0 mm. below the dorsal surface of the cord, just medial to the root entry line. A comparison of record 5A, in which no stimulus was given, with 5B, in which a single pyramidal shock was delivered, indicates clearly that a single pyramidal volley does not precipitate activity of the intermediate type. It is only in record 5D, obtained with the use of three pyramidal shocks, and in the subsequent records of Fig. 5, that unmistakable activity of intermediate type is to be found. The fact that several pyramidal shocks are neces-

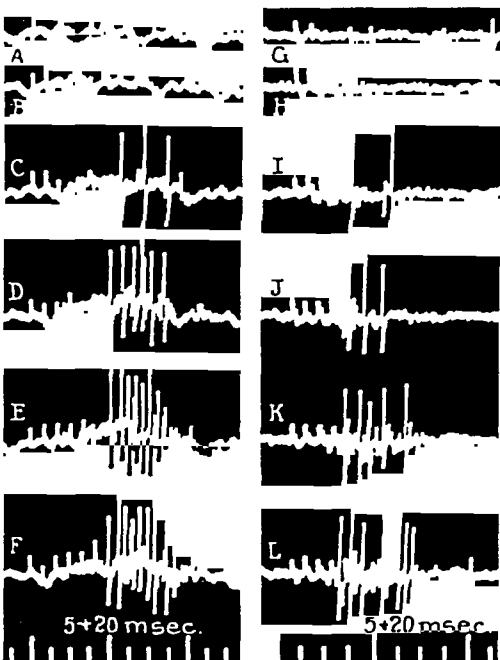


FIG. 6. Activity in the intermediate region resulting from one to six pyramidal shocks. A-F recorded from the lumbar cord. G-L recorded from the cervical cord. Note the difference in total latency for activation of the intermediate regions of cervical and lumbar enlargements under otherwise similar conditions, also background discharges of low amplitude.

sary to bring about the intermediate type activity has been regularly and repeatedly confirmed. Other examples of this observation may be found in Fig. 6A to F, recorded again from the lumbar enlargement, and in Fig. 6G to L, recorded from a similar region in the cervical enlargement. It is of particular interest that several pyramidal volleys are required to obtain an intermediate type discharge in the cervical cord, for there dispersion of the pyramidal tract volleys is so much less (compare Fig. 2B with Fig. 2C, D,

and E). It may be inferred that dispersion of the pyramidal impulses *per se* is not the primary cause of the failure of the intermediate region to respond to single pyramidal volleys.

A comparison of the activity characteristic of the intermediate region with that most prominent at the external basilar region (Fig. 3) shows that the former only occurs under conditions which are known to intensify the latter, and furthermore, that the former begins later and ends earlier than the latter under similar durations of pyramidal stimulation. A study of Fig. 5 and 6 reveals that the intermediate type activity appears on a background of fine discharges which are in no fundamental sense different from those in the external basilar region. The relationship between small cell activity (external basilar and intermediate) and the intermediate type activity is such as to lead to the conclusion that the intermediate type units become active largely as the result of discharges projected from the small cell elements. This conclusion does not exclude the possibility of direct synaptic connections from pyramidal fibers. Connections of the latter sort must in fact be considered for the following reason (cf. Fig. 7).

In the responses presented so far there has been no indication that units of the gray substance follow the frequency of the pyramidal stimulus. This may not be cause for surprise, since dispersion is great and the stimulation frequency high (cf. Bronk, Pitts, and Larrabee, 1940). Figure 7, however, shows a single experiment in which a unit was found to follow exactly the stimulation frequency. The micro-electrode was placed in the intermediate region (2.1 mm. down, 0.5 mm. medial to the root entry line). In each record of Fig. 7 (all similar) some of the responses are dropped. The dropped responses are the fifth, seventh, and eighth in A; the fourth and seventh in B; and the sixth and eighth in C. Discounting the dropped responses, the remainder are absolutely fixed with respect to the causal pyramidal shocks. The facts (i) that shortening of the pyramidal pathway to this unit does not take place (cf. discussion in connection with Fig. 11), and (ii) that some elements of the intermediate gray substance can be driven at the (high) frequency of the pyramidal volleys indicate a rather close anatomical connection between pyramidal fibers and such intermediate elements.

Facilitation of motoneurons by pyramidal action. The two-neuron-arc re-

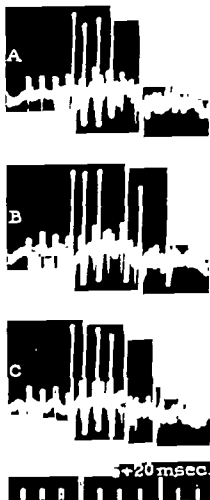


FIG. 7. Example of nuclear elements of the intermediate region following exactly the pyramidal stimulus frequency. As the frequency is high, a fairly close relationship between tract fibers and these nuclear elements is indicated.

flex discharge described by Renshaw (1940) has proved of value for the purpose of testing the average excitability of a segmental population of motoneurons. Since the motoneuron discharge that results from short trains of pyramidal shocks cannot be measured with any degree of accuracy, it has been necessary to rely upon alterations in testing two-neuron-arc reflex discharges for a reliable gauge of pyramidal influence over the motoneurons.

Figure 8 shows plotted curves constructed from an experiment in which the influence, on the two-neuron-arc discharge, of one to six pyramidal shocks was examined. The coordinates show the amplitude of the two-neuron-arc discharge as a function of time after the onset of the pyramidal stimulation. A single or two pyramidal shocks (curves 1 and 2) had no effect on the motoneurons that could be detected by the method. Three shocks resulted in a brief period of facilitation in the motor pool, after a total latent period of approximately 12 msec. The total latent period for facilitation at the motoneuron level varies from 12 to 20 or more msec., its duration being, in part, a function of the frequency of pyramidal stimulation (compare the motoneuron facilitation curves of Fig. 9A and B). Figures 9, 10, and 11 show examples from other experiments of the total latency for motoneu-

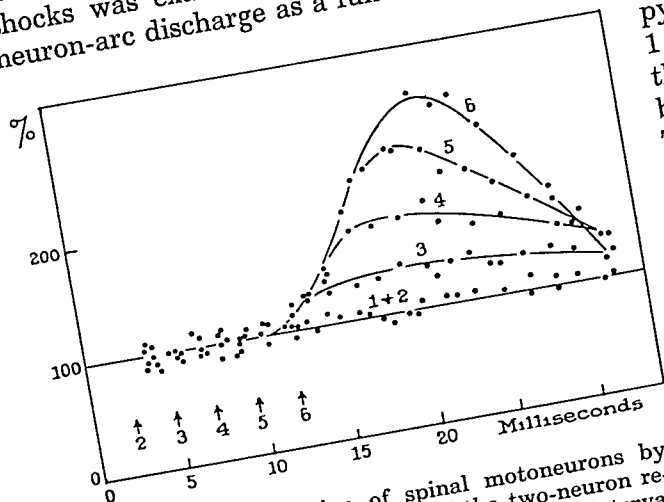


FIG. 8. Facilitation of spinal motoneurons by pyramidal activity. Amplitude of the two-neuron reflex response (ordinates) is plotted against the interval at which the reflex follows the single pyramidal shock, or first pyramidal shock of a train. The numbered arrows indicate the timing of the pyramidal shocks. The number of each curve of the family identifies that curve with the number of pyramidal shocks employed in the conditioning stimulation. Amplitude of the test response in isolation is 100.

ron facilitation. The values found in these experiments were approximately 14 and 18, 15.5, and 12 msec. respectively.

As more pyramidal shocks are added to the train, facilitation of the motoneurons increases in intensity (Fig. 8, curves 4, 5, and 6). The duration of the facilitation period due specifically to the sixth pyramidal shock (obtained by subtracting the area enclosed by curve 5 from that enclosed by curve 6) is much shorter than is the duration of the facilitation period specifically related to the third shock (area enclosed by curve 3). Thus, to parallel the increase in intensity there is a more rapid summation of subnormality (Gasser, 1935; Lorente de Nó and Graham, 1938) that occurs at the pre-motoneuron level rather than at the motoneuron level itself, for the feeble motoneuron discharge is not likely to evoke significant motoneuron subnormality. On

this interpretation, the more rapid failure of pre-motoneuron activity consequent upon more intense activation would increase reflex threshold in the ventral horn secondarily by diminished opportunity for summation there.

A comparison of facilitation in the motor nucleus (Fig. 8, also Fig. 9, 10, and 11) with discharges recorded from the intermediate region of the gray substance (Fig. 5 and 6) shows that the two events are closely parallel. Given anatomical connection (Kölliker, 1890; Cajal, 1909; and others), the parallelism appears to establish a causal relationship. The conclusion may be reached that activity in the intermediate region, following pyramidal stimulation, is the principal contributing factor to excitation in the motoneuron pool. The solitary cells of the dorsal horn, as stated above, may form additional links between the pyramidal fibers and the motoneurons.

Facilitation of reflex arcs at internuncial levels. Just as the two-neuron-arc discharges have proved of use for testing the excitability of motoneurons three-neuron-arc reflex discharges now prove to be equally useful in detecting average excitability changes at the internuncial level, when evaluated in terms of alterations in discharges mediated through arcs of two neurons. In theory, three-neuron-arc reflex discharges may be facilitated at two points; at the junction of primary afferent neurons with interneurons, and at the junction of interneurons with motoneurons. If, however, a three-neuron-arc discharge be facilitated in the absence of any change in the concomitant two-neuron-arc discharge, it may be said that the facilitation has occurred at the internuncial level.

Figure 9 illustrates the effect of pyramidal stimulation on reflex discharges pertaining to arcs of two and three neurons. The inset B of Figure 9 shows the dorsal to ventral root reflex discharge resulting from a single

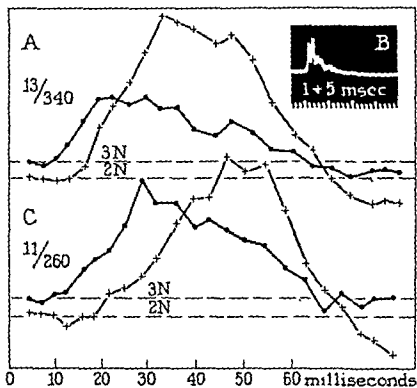


FIG. 9. Facilitation of two-neuron and three-

neuron arcs. The amplitudes of the two-neuron response (crosses) and three-neuron response (circles) are plotted in A and C as functions of time after the first shock in a train of pyramidal shocks. The hatched lines, 3N and 2N, of A and C show the amplitude of the test responses in isolation. In A, 13 shocks at 340 per sec. provided the conditioning activity. In C, 11 shocks at 260 per sec. were used for conditioning. Full description in text.

shock. The first two clearly defined spike potentials, separated in time by approximately 0.7 to 0.8 msec., are the two and three-neuron-arc discharge spike potentials respectively. It is the amplitudes of these two spike potentials that are plotted in Fig. 9A and C, as a function of time after the onset of the pyramidal stimulation. In Fig. 9A, 13 shocks were delivered to the pyramid at a frequency of 340 per sec.

Facilitation of the two-neuron-arc response in Fig. 9A (crosses) begins approximately 14 msec. after the onset of the pyramidal stimulation. In contrast, facilitation of the three-neuron-arc reflex discharge (circles) begins several milliseconds earlier, at about 9 msec. after the first pyramidal shock. Thus for a period of approximately 5 msec. the three-neuron-arc may be regarded as being facilitated only at the internuncial (pre-motoneuron) level. A time differential amounting to as little as 3 msec. between the facilitation of three-neuron and two-neuron-arc discharges has been encountered. The total latency for facilitation of three-neuron-arc discharges varies between 9 and 12 msec. *Because the beginning of the solitary cell discharge (Fig. 4) falls within the 9 to 12 msec. time range, it is possible that the solitary cells supply impulses by collaterals to the interneurons occupied by the three-neuron-arc discharges.*

As the facilitation of two-neuron-arc discharges progresses, there is a secondary decrease in the amplitude of the facilitated three-neuron-arc discharge, which undoubtedly indicates that a strongly facilitated two-neuron-arc discharge occupies more of the motoneurons that would otherwise be at the service of three-neuron arcs, *i.e.* the facilitated two-neuron-arc discharge occludes the succeeding three-neuron-arc discharge (the law of plurality of connections, Lorente de N6, 1933, 1938).

The view that two and three-neuron-arc discharges have some degree of reciprocal relationship is strengthened by a consideration of the curves presented in Fig. 9C. Figure 9C is similar to Fig. 9A, with the single exception that 11 shocks were delivered to the pyramid at a frequency of 260 per sec. With the slower frequency of stimulation employed in Fig. 9C, motoneuron facilitation (crosses) begins later (about 18 msec. after the first pyramidal shock) and progresses more slowly to reach a later maximum. Facilitation at the internuncial level also begins later and progresses more slowly. However, the added delay in facilitation is greater at the motoneuron level than at the internuncial level, with the result that the three-neuron-arc discharge may develop further before measurable occlusion begins by virtue of facilitated two-neuron-arc discharges.

In some experiments the two-neuron-arc discharge is so completely dominant in the unisegmental reflex that it becomes impossible to analyze events in higher order chains. Conversely, if a bisegmental reflex is substituted for the unisegmental reflex as a test system, an opportunity arises to study the behavior of three-neuron arcs relatively unencumbered by strong two-neuron-arc discharges. Figure 10 presents records from an experiment in which the bisegmental reflex discharge (10B) is conditioned at various time intervals

by 6 pyramidal shocks (10A). The earliest two-neuron-arc discharge occurs in 10H. Between H and O of Fig. 10, two-neuron arcs are facilitated, and presumably less direct reflex pathways also can be facilitated at the motoneuron level. Observations D, E, and F show that delayed segments of the reflex discharge are facilitated before the onset of facilitation at the motoneuron level. In 10G, the three-neuron-arc spike potential (still the initial spike potential of the reflex) is just overlapping the beginning of the period

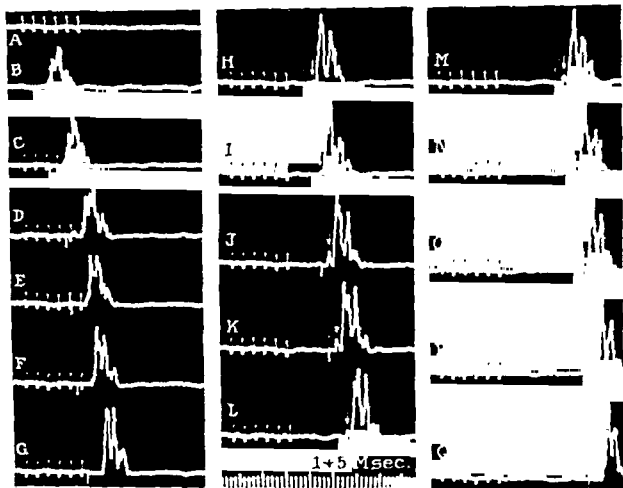


FIG. 10. Effect of six pyramidal shocks on bisegmental reflex discharge (L7-S1). Recording leads on S1 ventral root. A, response to six pyramidal shocks. B, response to testing L7 dorsal root shock. In C to Q, the test dorsal root shock falls progressively later with respect to the pyramidal train. Two-neuron-arc discharges are identified by arrows.

during which facilitation is known to occur at the motoneuron level. The two-neuron-arc facilitation is maximal in 10J, but even so it is so small that it does not interfere measurably with facilitation of the three-neuron-arc discharge. Occlusion, which is a prominent feature in Fig. 9, is not detectable in Fig. 10, in which the two-neuron-arc discharge is small. This fact again points to the multiplicity of connections between primary afferent collaterals and spinal motoneurons.

In Fig. 10 P and Q are of interest because they show, in the absence of occlusion at the motoneuron, that higher order reflex discharges (conspicuously the three-neuron-arc discharge) are strongly facilitated after facilitation in the two-neuron-arc is no longer in evidence. The period during which

facilitation at the internuncial level of the three-neuron-arc, including certainly the intermediate gray nucleus of Cajal, may be demonstrated, by the use of bisegmental testing reflex discharges, parallels the discharge period of the external basilar region. Undoubtedly the two events are related. Furthermore, Fig. 10 shows that the internuncial level of the three-neuron-arc receives facilitating impulses for a longer period of time than it discharges impulses in turn to the motoneuron pool. Thus the evidence of Fig. 10 agrees closely with that of Fig. 8, employing similar conditions of stimulation in another preparation, and again points to the intermediate region (internuncial level of the three-neuron-arc) as the locus at which failure of the spinal mechanism to transmit pyramidal excitatory action first occurs.

The time interval between the onset of facilitation of the three-neuron arcs and of the two-neurons arcs is a measure of the time between the beginning of impulse arrival in the intermediate gray nucleus, and the beginning of impulse arrival in the motoneuron pool. Since the discharge of impulses in the intermediate region coincides with the arrival of impulses in the motoneuron pool, and since the two groups of neurons are synaptically related, the time difference between facilitation of three and two-neuron-arc discharges (Fig. 9 and 10) may be taken as a measure of the "nuclear delay"*

Since the total latency for excitation at the motoneuron level is so long, it becomes of interest to determine if possible the shortest functional pathway that may be established by later volleys in a pyramidal tetanus, working on the background of activity supplied by antecedent volleys. An estimate may be reached by measuring the latency from a specific shock in the train of shocks to the onset in the motor nucleus of an effect attributable to that shock. For example, the onset of facilitation in Fig. 8, after 12 msec. total latency, is in part the result of the application of the third pyramidal shock 4.8 msec. after the initial shock of the series (Fig. 8, curve 3). This is the case since the first two pyramidal shocks had no tangible effect on the motoneurons (Fig. 8, curves 1 and 2). The resulting specific latency of the specific shock amounts to approximately 7.2 msec. An accurate estimate of the specific latencies for the fourth, fifth, and sixth shocks in the experiment illustrated in Fig. 8 is not possible. Figure 11, therefore, presents curves from another experiment, which embody many more individual observations. As in Fig. 8, two pyramidal shocks had no measurable effect on the motor pool. The third shock of the train produced, by intensification and spread, a period

* Nuclear delay may be defined as the observed discrepancy in time between the arrival of impulses in a nucleus and the discharge of impulses from that nucleus. Thus it might be the equivalent of the recruitment period or even of a utilization period; it could not be the equivalent of synaptic delay (cf. Lorente de Nó, 1935). Nuclear delay is a function of the presynaptic elements of the nucleus because, when the intermediate nucleus is activated (i) through the pyramidal system, nuclear delay amounts to 3 or more msec.; but when activated (ii) through primary afferent collaterals, nuclear delay can occupy no more than a fraction of a msec. A unique and definitive account of the significance of nuclear delay as determined above is not apparent from the determinations themselves.

SPINAL MECHANISM OF PYRAMIDS

of facilitation in the motor pool beginning at A. The horizontal displacement of arrow 3-A denotes the specific latency between the third shock and its effect in the motor pool (approximately 7.3 msec.). On the graph are shown the facilitation curves resulting from 5 pyramidal shocks (circles) and from 6 pyramidal shocks (crosses). Since the two curves are superimposed, the point of divergence indicates the time at which the effect of the sixth pyramidal shock is first exerted on the motoneurons. Had the sixth shock main-

tained the same specific latency as that of the third, the facilitation curves of Fig. 11 would diverge at B, since the horizontal displacement of arrow 6-B was drawn to equal that of arrow 3-A. Such is obviously not the case. The specific latency for the sixth shock is approximately 1.5 to 2 msec. shorter, i.e. 5.3 to 5.8 msec. in duration. Of this specific latency 4 to 4.5 msec. is attributable to pyramidal tract conduction, assuming the more rapidly conducting tract fibers to be involved. The remainder (0.8 to 1.8 msec.) is lost in the spinal mechanism, and it is of such time dimension as to suggest indirect pyramidal action on the motoneurons through internuncial relays. Since the present argument is based largely on time relationships, it will be seen that it is not necessary to assume the intercalation of interneurons between the more slowly conducting pyramidal fibers and the motoneurons. The action on motoneurons of direct pyramidal connections, if they exist, must be small, for it has not been detected by the use of single pyramidal volleys.

The discharge of motoneurons in response to pyramidal stimulation. In many of the experiments of the present series a regular tonic discharge of impulses from the motoneurons has been detected readily by means of recording leads placed on a ventral root (Fig. 12A). Tonic innervation is well-known in the decerebrate preparation (decerebrate rigidity—Sherrington 1897; cf. Denny-Brown, 1929; Adrian and Bronk, 1929). However, the tonic discharge of spinal motoneurons under the conditions of the present experi-

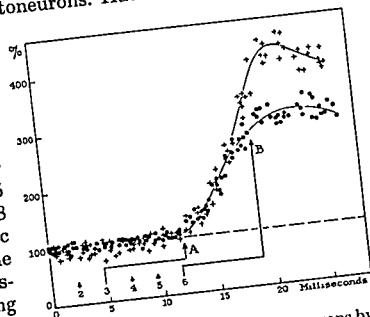


FIG. 11. Facilitation of motoneurons by 5 pyramidal shocks (circles) and 6 pyramidal shocks (crosses). The curves are constructed as in Fig. 8, the two-neuron-arc discharge again being used as a test. The arrows numbered successively at the bottom indicate the timing of the pyramidal shocks. The interval between the time of the sixth shock and the point of divergence of the two facilitation curves denotes the specific latency between the sixth shock and its effect in the motor pool. Arrow 3-A denotes the specific latency for the third pyramidal shock. Arrow 6-B has the same time span as Arrow 3-A. Note that the curves diverge before B; hence there is progressive shortening of latency in the spinal mechanism.

ments is not a manifestation of decerebrate rigidity, for the vestibular and reticular pathways are severed (cf. Fulton, Liddell, and Rioch, 1930a, b), nor can it be due to maintained discharges from the cerebral cortex (Adrian and Moruzzi, 1939), because the brain stem is transected routinely at the colliculi. There remains the continuous flow of afferent impulses entering through the dorsal roots, previously mentioned. The tonic afferent discharge is probably the major factor in maintaining the tonic motoneuron discharge in the spinal animal. The tonic motoneuron discharge seen in these preparations is probably related to the flexor rigidity of Dusser de Barenne and Koskoff (1932, 1934). It may be noted that the spinal clamps that have been used

in these experiments hold the preparation in a position appropriate for the development of flexor rigidity.

A prolonged* pyramidal tetanus (245 msec. duration) evokes after 20 to 30 msec. latency a discharge of motoneurons which takes the form of an increase in the level of the pre-existing tonic discharge (Fig. 12B). The added discharge often shows a maximum at 35 to 50 msec., following which is a depression and a secondary increase: After the cessation of the pyramidal stimulation, the duration of which is indicated by the horizontal line immediately below record 12C, the motoneurons "afterdischarge" for several hundred milliseconds.

It is difficult to estimate the part played by the pyramidal system *per se* in these prolonged phenomena, for once movement begins, locally engendered activity, reflecting from the periphery, may supervene to reinforce the pyramidally

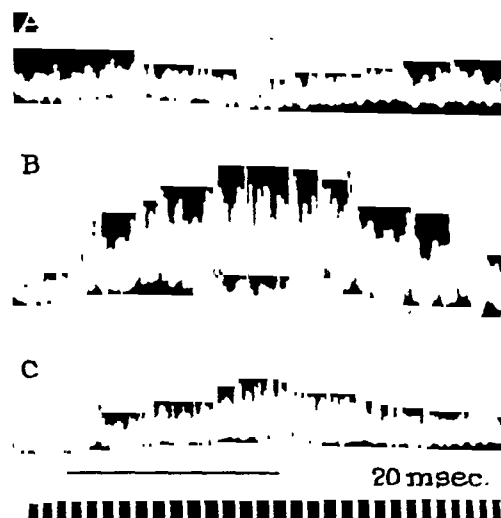


FIG. 12. Records from ventral root. A, tonic discharge of motoneurons (spinal preparation). B, motoneuron discharge resulting from pyramidal stimulation of 245 msec. duration. C, and B, but after prevention of movement by curare. Note afterdischarge in B and C. The horizontal line below C indicates the duration of the pyramidal stimulation in B and C. Further details in text.

evoked activity. For instance, after record 12B was taken curare was administered to the preparation until movement was no longer observed. Figure 12C illustrates the discharge of motoneurons obtained by the pyramidal tetanus in the paralyzed preparation. The tonic activity is less, as is the activity evoked by the pyramidal stimulation. Note, however, that the

* These stimulations are not prolonged in the sense usually accepted in studies on the pyramidal system, nor are the frequencies employed throughout comparable with those usually employed. The reason for this of course is the purpose underlying the experiments, which likewise is different.

motoneurons still maintain an afterdischarge for several hundred msec. in the curarized preparation. A comparison of Fig. 12B and C demonstrates in an elementary way the reinforcing action of secondary peripherally evoked activity.

Inhibition. Inhibitory actions frequently have been attributed to the pyramidal tract. Figure 13 presents one of several instances in which activity recorded from the intermediate gray substance was suspended rather than initiated or intensified by pyramidal stimulation. Record A of Fig. 13 illustrates the activity which was recorded in the absence of specific stimulation.* Record B of Fig. 13 shows the suspension of activity brought about by a pyramidal tetanus of approximately 100 msec. duration. Other units will be brought into activity during the time that the units at present under discussion are "inhibited" (cf. Fig. 5 to 7). Granting anatomical connection between the "inhibited" units and the motoneurons, the motoneuron discharges of Fig. 12 represent the final resultant of simultaneously occurring excitatory and inhibitory actions (direct or indirect, Lloyd, 1941b). It is not possible from the present experiments to determine whether or not the operation of reciprocal innervation, herein demonstrated for interneurons, is maintained within the motor pool by specific distribution of internuncial axons. The observations of Fig. 13 throw no light on the nature of the fundamental processes involved, which is in consequence not discussed.

Functional organization of the spinal mechanism of the pyramidal tract. Figure 14 presents in diagrammatic form a summary of functional connections between fibers of the pyramidal system, primary afferent collaterals, and neurons of the spinal cord, as they appear in the light of the foregoing observations. The diagram is admittedly incomplete and imperfect. The pyramidal fibers (P) are pictured as ending most prominently on the cells of the external basilar region (E). Possible connections to the solitary cells (S) and some cells of the intermediate region are indicated. There is little indication from the present experiments that pyramidal fibers end on motoneurons (M.N.). In consequence no connections



FIG. 13. Inhibition of tonic interneuron activity by pyramidal activity, (100 msec. tetanic stimulation). Note the different times at which various elements return to activity.

* One is inclined to accept the physiological nature of the internuncial discharges "spontaneously" occurring in Fig. 13, for they may continue unabated for hours with amplitude and phase relationships of the spike potentials preserved. Likewise, since the preparations exhibit a constant afferent inflow and a tonic motoneuron discharge, there is no reason to suppose that the interneurons would not display activity if the recording microelectrode were not in position.

are included in Fig. 14. The cells of the external basilar region (E) relay activity to the solitary cells (S) and to the intermediate region (I and I₂). The intermediate gray nucleus of Cajal is represented (I). Primary afferent collaterals (P.A.) convey activity through branches 3 and 3a to interneurons, which in turn supply motoneurons (M.N.) thus completing three-neuron reflex arcs. Furthermore, primary afferent collaterals (P.A.) through branch 2 to the motoneurons (M.N.) complete the two-neuron reflex arcs.

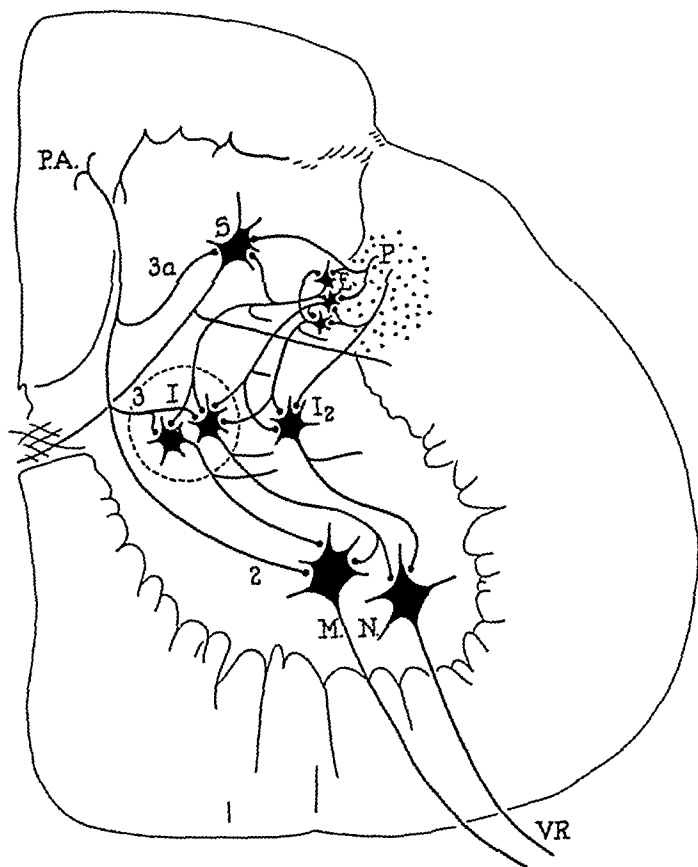


FIG. 14. Functional organization of the spinal mechanism of the pyramidal tract. Connections from the pyramidal tract and primary afferent collaterals are represented. E, small cells of the external basilar region; I, intermediate gray nucleus of Cajal; I₂, other neurons of the intermediate region; M. N., motoneurons; P, pyramidal tract and fibers; P. A., primary afferent collaterals; S, solitary cells of the dorsal horn; V. R., ventral root; 2, 3, and 3A, terminal collaterals of the primary afferent system.

Emphasis must be placed on the fact that activity within the spinal gray substance is continuous from the arrival of the first tract impulses as the result of pyramidal stimulation until the spinal mechanism reaches the highest activity level possible under the conditions of the experiment. Within the stroma of continuous activity certain neurons, or groups of neurons, become

active at well defined and reproducible time intervals after the onset of the pyramidal stimulation. The dynamic characteristics of the spinal mechanism of the pyramidal tract may be summarized by a brief recapitulation of two type experiments. In the first type experiment a single pyramidal volley is used. Pyramidal impulses first enter the lumbo-sacral cord at 4.5 msec. after the pyramidal shock and continue to do so for possibly 10 or more milliseconds (Fig. 2). They are accompanied by a burst of nuclear activity in the external basilar region (Fig. 3). Other types of internuncial activity do not ensue, nor is reflex activity affected demonstrably throughout the activity period. Presumably following a single pyramidal shock, the impulses of the external basilar region (E in Fig. 14) circulate within the nucleus itself, extranuclear discharges through longer collaterals to other nuclei being negligible. In the second type experiment several pyramidal shocks are used. The minimal latency of pyramidal conduction is 4.5 msec. as before, but tract activity and external basilar region activity are intensified by the added pyramidal shocks. The contribution of impulses to other internuncial nuclei becomes sufficiently great to result in measurable facilitation at the internuncial level of the three-neuron reflex arc at 9 msec. after the onset of the pyramidal stimulation. Thus approximately 4.5 msec. are lost in the dorsal horn. An additional 3 msec. are lost in nuclear delay at the intermediate region before intermediate neurons discharge and motoneurons are facilitated. Thus the total latent period for facilitation of motoneurons (12 msec.) is accounted for by (i), tract conduction (4.5 msec.); (ii), dorsal horn latency (4.5 msec.); and (iii), nuclear delay at the intermediate level (3 msec.)

As activity spreads slowly through the spinal gray substance, progressively shorter functional pathways are opened to the pyramidal impulses, so that the time lost in the internuncial pools of the spinal gray substance is reduced from approximately 7.5 msec. to approximately 1.0 msec. (Fig. 11).

When pyramidally evoked activity reaches the motoneurons, the motoneuron discharge realized through three-neuron reflex arcs may be facilitated at both the interneuron and motoneuron levels. If a large two-neuron-arc response is present in the test reflex discharge, occlusion of the three-neuron-arc discharge at the motoneuron level counteracts the facilitatory action of the pyramidal stimulation.

SUMMARY

A method is described whereby a controlled pyramidal volley may be delivered into the spinal cord. Using this method, an attempt is made to outline the functional organization of the spinal mechanism under the conditions of pyramidal stimulation. A summary of this organization is presented in connection with Fig. 14. Cats were used.

Pyramidal activity is distributed along the length of the spinal cord by the pyramidal tract fibers. The most rapidly conducting of these have a velocity between 60 and 65 M per sec. The lower limit of pyramidal fiber velocities is uncertain, but dispersion of a volley, initially synchronous at

the medullary level, is sufficient to produce a discharge of pyramidal impulses into the lumbar cord lasting many milliseconds.

Interneurons of the spinal cord are of paramount importance in effecting the distribution of pyramidal activity. Small nuclear elements, in close proximity with the tract, appear to constitute the initial internuncial relays. The final or premotoneuron internuncial relays lie within the intermediate region. Facilitation of motoneurons, as tested through primary afferent volleys, parallels activity in the intermediate region. In view of known anatomical connections a causal relationship between intermediate internuncial discharges and excitation of motoneurons appears to be established.

A nuclear delay of several milliseconds exists at the intermediate nucleus under the conditions of pyramidal excitation. The same nucleus activated through primary afferent collaterals has a nuclear delay of but a fraction of a millisecond. The differences are a function of the presynaptic elements rather than of the postsynaptic elements.

As pyramidal activity is intensified, shortening of the functional paths interposed between pyramidal fibers and motoneurons occurs. The rate of shortening is related to the frequency of pyramidal stimulation.

Whereas some interneurons of the intermediate region are activated by pyramidal stimulation, others are inhibited (reciprocal innervation). Tonic discharge of spinal motoneurons occurs in the spinal state. This discharge is probably related to the flexor rigidity of Dusser de Barenne and Koskoff. The constant arrival of impulses from the periphery must be considered as an important factor in the maintenance of the tonic discharge in the spinal preparation.

Motoneuron discharge resulting from pyramidal activity is highly asynchronous, although without doubt some motoneuron units carry the pyramidal stimulus frequency.

Spinal reflex reinforcement of pyramidal activity begins with the onset of movement, and it may be important in determining the character of motor performance arising from cortical stimulations.

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CROSSED INHIBITION OF THE FLEXOR REFLEX IN THE SPINAL MAMMAL*†

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INTRODUCTION

CURRENT theories of inhibition may be divided into two groups. The first refers the depression of irritability to immediately preceding activity of the inhibited units; the second usually invokes specific inhibitory endings. The data presented here afford instances which cannot be explained upon the former hypothesis and accord well with the latter. In a paper published in March, 1941, Lloyd (6) reached identical conclusions from a different approach. Both researches dealt with anterior horn cells. Lloyd employed cats under Dial, we, spinal monkeys free from anesthesia at the time of recording. The independence of the two lines of evidence strengthens the argument for this class of inhibition. Its occurrence in two such divergent species affords strong support for the general application of the cases considered.

The present communication is not confined, however, to the problem of the nature and locus of crossed inhibition. Since a tenable inhibitory theory of the genesis of spinal shock has recently been advanced by Van Harreveld (16), this occasion would seem pertinent for the discussion of certain differences between inhibition curves obtained within a few hours of spinal transection and those recorded weeks after that operation. In the interest of clarity, the presentation of results opens with this phase of the subject.

RESULTS

Interaction of two successive volleys to roots or nerves of opposite sides has been studied in 24 cats, 6 dogs, and 30 monkeys. Of these, 14 cats, 4 dogs, and 8 monkeys were recorded on the day the cord was severed; 10 cats, 2 dogs and 22 monkeys at intervals ranging from 3 to 64 days after transection. Cord potentials were recorded from leads on the surface of the cord close to the entrance zone of the posterior roots at an amplification of 15 mm. per mV. Reflexes were recorded by isometric levers or in some cases electrically from a lead in the muscle.

Inhibition in the cat. Individual variations were too great to justify statistical treatment of the data. A few acutely spinal cats yielded inhibition curves of chronic pattern. We have never recorded an acute type of curve from a chronic cat. The dictum of Hughes and Gasser (2) that in the acutely spinal cat the presence of inhibition is associated with a positive wave in

* These results were reported at the Philadelphia Physiological Society, October 15, 1940 (8).

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the cord potential has proved valid for crossed as well as for ipsilateral inhibition (3) and for dogs and monkeys as well as for cats.

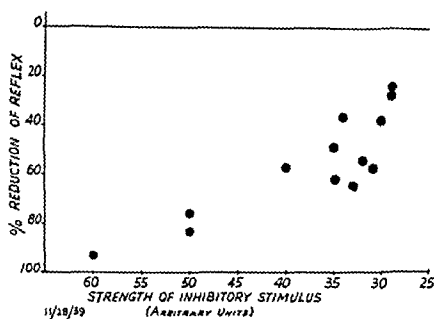


FIG. 1. Relation of strength of crossed inhibitory shock to degree of reflex inhibition at a constant interval of 46 msec in a cat 4-6 hr. after transection of the spinal cord. Excitatory shock to right sciatic nerve, constant strength (40 units). Inhibitory shock to left sciatic nerve; strengths indicated on abscissa. Ordinate indicates percentage reduction in contraction of right semitendinosus muscle. 11/28/39.

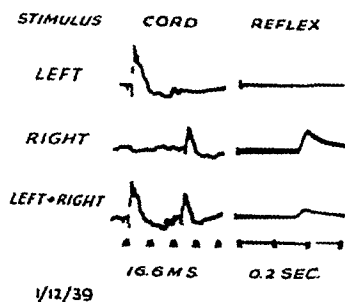


FIG. 3. Crossed inhibition of the flexor reflex without reduction of cord potential. *Macaca mulatta* 59 days after transection. 1/12/39. Stimulation of left and right peroneal nerves. Tension record from semitendinosus muscle.

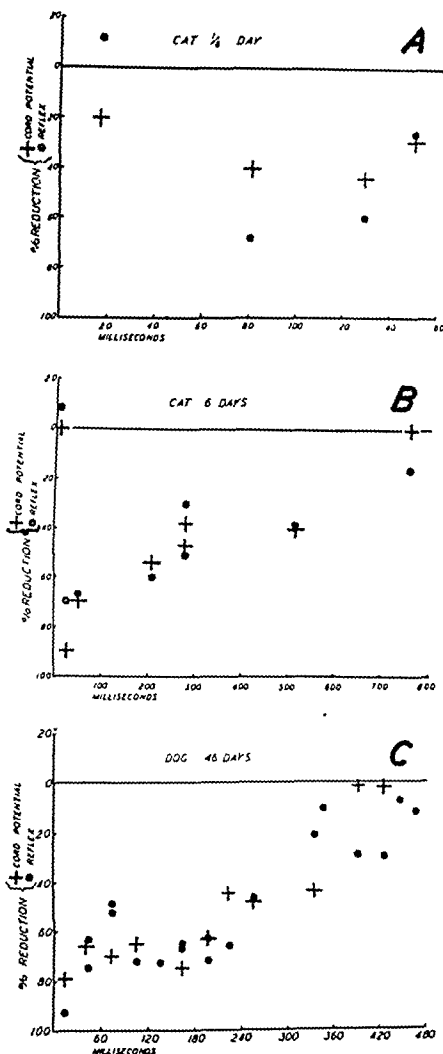


FIG. 2. Recovery curves of cord potential (plus signs) and reflex (dots) from crossed inhibition at various intervals after spinal transection: (a) cat 4 hours after transection; (b) cat 6 days after transection; (c) dog 46 days after transection.

In all three species the prominence of the positive wave of the cord potential (15) and the depth and duration of inhibition increase with the interval after transection. Thus in the acute cat a flexor reflex evoked by a

stimulus of twice reflex threshold strength is commonly inhibited by a crossed volley of four times threshold strength to a maximum depth of about 50 per cent of the test contraction, the inhibition persisting for less than a fifth of a second. In the chronic animal a conditioning volley of half this strength commonly induces complete inhibition which is still demonstrable after an entire second.

If inhibition be obtainable in the acutely spinal cat, it occurs after an inhibitory volley confined to alpha, beta, and gamma fibers. Occasionally a

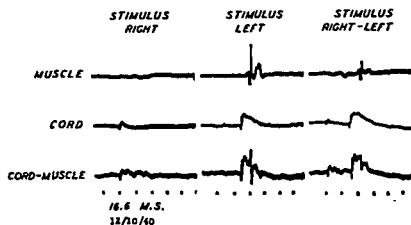


FIG. 4. Crossed inhibition of the flexor reflex without reduction of cord potential. *Macaca mulatta* 6 hr. after transection. 12/10/40. Top row, ground lead in semitendinosus muscle. Middle row, ground lead on cord. Bottom row, ground lead on cord, grid lead in semitendinosus; stimulating right and left posterior roots.

pronounced crossed inhibition of the flexor reflex may be conditioned by a stimulus scarcely above the threshold of the cord potential, involving only the fibers of lowest threshold in the alpha group. Within wide limits, the stronger the inhibitory shock, the deeper and more prolonged is the inhibition (14). The depth of inhibition at a constant interval of 46 msec. is plotted against the strength of the conditioning shock in Fig. 1.

In a previous paper (3) the parallelism in inhibition of the internuncial cord potential and the reflex response was emphasized. It is only in the chronic animal, however, that the two curves are identical. Acute cats usually show a somewhat deeper inhibition of the reflex response than of the cord potential (Fig. 2a). Though inhibition at the internuncial level accounts for the major share of reflex inhibition, this divergence of the two curves affords evidence in the acute preparation of some inhibition farther downstream.

Another feature of the pattern in the acute cat is facilitation at short intervals giving way at about 20 msec. to progressively deepening inhibition which reaches its greatest intensity between 30 and 60 msec. With increasing

chronicity this initial facilitation is curtailed and ultimately disappears (Fig. 2b and c). We are inclined to associate it with the crossed flexor reflex which recovers from spinal shock earlier than crossed extension only to suffer progressive eclipse as the latter reflex emerges from depression (9). These effects

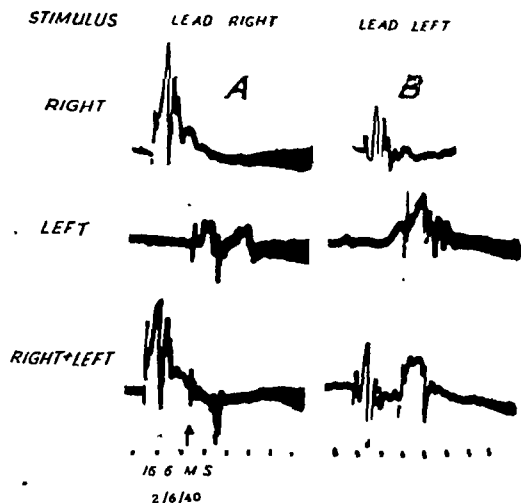
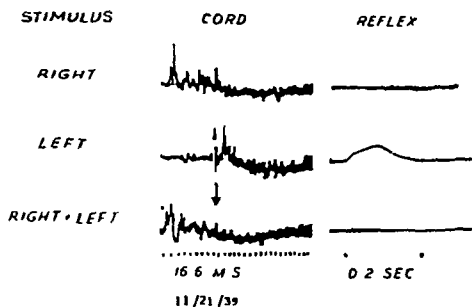


FIG. 5. Difference in the degree of crossed inhibition of the potential set up by a test volley in right and left dorsal horn regions respectively. *Macaca mulatta* 6 hours after transection. 2/6/40. Conditioning stimulus, right dorsal root, test stimulus, left dorsal root. (A) When the lead is from the dorsal horn on the same side (right) as the conditioning stimulus. (B) From the dorsal horn on the opposite side (left) to the conditioning stimulus

FIG. 6. Complete inhibition of cord potential and reflex, in *Macacus mordax* 36 days after transection. Excitatory stimulus, left external cutaneous nerve. Inhibitory stimulus, right external cutaneous nerve. Left semitendinosus muscle. 11/21/39.



of recovery from spinal shock are illustrated in the recovery curves comprising Fig. 2.

Inhibition in the monkey. In dog and monkey crossed inhibition is elicited only by stimuli far stronger than those which are effectual in the cat. In many acute monkeys it is unobtainable whatever the strength of the conditioning stimulus. Even in the chronic animal it is only by activating an appreciable number of delta fibers that we have obtained consistent results. In this regard, crossed inhibition differs from ipsilateral inhibition (10). It

reflects the increasing severity of spinal shock with ascent from lower to higher mammals.

A more important difference is in the stability of the internuncial cord potential. An instance in a chronic monkey of reflex inhibition without reduction in the internuncial potential has previously been reported (10) and is here reproduced as Fig. 3. In the acute monkey this is a not infrequent result (Fig. 4) and cases of deep reflex inhibition associated with only slight reduction of internuncial potential are common. In these with suitable grading of inhibitory and excitatory volleys, the crossed component of the internuncial potential from the excitatory volley proves to be more susceptible to inhibition than does the ipsilateral component (Fig. 5). It is only in the chronically spinal monkey in good condition that we have obtained reduction of corresponding degree of internuncial potential and reflex by a strong conditioning volley (Fig. 6 and 7).

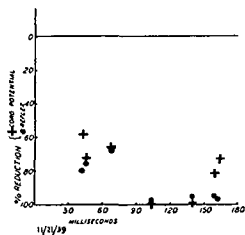


FIG. 7. Recovery curve indicating comparable degree of inhibition of cord potential and reflex in experiment illustrated in Fig. 9.

DISCUSSION

Van Harreveld (16) has recently reported that a brief period of ischemia of the spinal cord below the level of transection is followed by a more rapid reflex recovery than is transection without ischemia. He suggests that spinal shock may be due to inhibitory reflexes released from the control of higher centers; that such reflexes are especially susceptible to ischemia, which serves, therefore, to release excitatory reflexes from their control. His experimental approach and theoretical deduction are ingenious and accord well with one conspicuous result of lower thoracic transection in the decerebrate: the enhancement of rigidity in the forelimbs, presumably due to release from a source of inhibition below the level of transection (13). If spinal shock be due to inhibition, excitatory reflexes must be presumed to be more susceptible to inhibition during the period of their depression than after their recovery. In the case of the knee jerk this is actually the case (5). With the ipsilateral flexor reflex, however, the reverse is true. This reflex is less susceptible to either contralateral (Fig. 2) or ipsilateral inhibition (4) during the earlier than during the later phases of its recovery. Hence we incline to question the inhibitory source of depression of this reflex following spinal transection. If we seek to explain the difference in susceptibility between the knee jerk and the flexion reflex, one factor is obvious. Whatever the mechanism of spinal shock, it depresses extensor reflexes more severely than flexor. The source of inhibition of flexors experimentally available is part and parcel of the extensor reflexes which are so deeply depressed and *vice versa*. Reciprocal inhibition is depressed *pari passu* with the excitatory component with which it

is associated. The system behaves as though one and the same axon that supplies excitatory terminals to an extensor motoneuron furnished inhibitory endings to a flexor motoneuron. The result appears to be determined more by the availability of the inhibitor than by the susceptibility of the perikaryon to inhibition.

On the other hand, these results offer one bit of evidence pointing to increased susceptibility of perikarya to inhibition during spinal shock. In previous papers evidence was advanced for the conclusion that in the monkey motoneurons suffer deeper depression after transection of the cord than do interneurons (15, 4), and crossing interneurons deeper depression than unilateral ones (10). During spinal shock the monkey's motoneurons are susceptible to crossed inhibition at a stage when the less depressed interneurons resist it. With further recovery, the internuncial potential is also inhibited by a crossed volley. Presumably, in the earlier phase the crossed inhibition is too weak to affect the interneurons of the dorsal horn but does involve the depressed motoneurons. Later the crossing neurons recover to the point where a sufficient number discharge to inhibit the cells of the dorsal horn as well. The greater susceptibility to inhibition of the motoneurons may well be due to their depression by spinal shock. Such an argument hinges upon the assumption of relatively equal distribution of inhibitory endings of crossing neurons to cells of the anterior and those of the posterior horn. Beyond the suggestion from Cajal's finding of division of crossing fibers into two branches, one passing to the anterior, the other to the posterior horn (11) evidence for such an assumption is still lacking. Even if enhanced susceptibility to inhibition be admitted as a feature of spinal shock, it is not sufficient in magnitude to dominate the recovery curve of flexor motoneurons. On the contrary, the progressive deepening of inhibition of the flexor reflex with recovery from spinal shock impresses us as contrary to expectation on the basis of Van Harreveld's theory. Yet it is far from disproving his suggestion. It does imply, however, that the reflexes he postulates are not dominantly concerned with inhibition of flexor units. Such a conclusion accords with the high extensor tone of his animals.

To turn briefly to another aspect of our results, the case of the monkeys in which crossed reflex inhibition occurs without demonstrable change in internuncial potential is of interest in relation to current theories of inhibition. Such theories may be divided into those which refer the reduction in irritability to immediately preceding response of the same units and those which invoke specific inhibitory endings. If absence of demonstrable change in the intermediary cord potential be accepted as evidence that excitatory drive upon the motoneurons is not essentially altered, then it implies that inhibition has occurred in them in the absence of immediately preceding activity.

Figures 3 and 4 exemplify this situation. In each the cord potential is led from an electrode about 1.5 mm. in diameter on the surface of the cord close to the posterior root entrance zone on the side of the reflex response and

recorded at an amplification of 15 mm. per mV. We believe these records indicate internuncial activity throughout the dorsolateral quadrant of the cord at the level of maximum response to the test volley with sufficient sensitivity to be altered by any significant change in excitatory drive from interneurons which it engages.* In both cases the record of the cord potential from the test volley when preceded by a contralateral shock is identical with that of the unconditioned control. In both there is significant inhibition of the reflex response. Hence inhibition has occurred at the motoneurons which was not conditioned by preceding activity of the units involved.

A more direct proof of the same conclusion is offered by Lloyd (6). With a micro-electrode lead from the anterior horn of the cat under Dial anesthesia he made use of Renshaw's finding (12) on the basis of latency measurements that the initial reflex discharge is set up in response to activity of afferents ending directly upon anterior horn cells. He was able to inhibit this initial volley by a conditioning shock to an adjacent dorsal root.

Hence there are now two independent proofs of inhibition of motoneurons which is not conditioned by preceding activity of the inhibited cells. Since such inhibition has been demonstrated in both cat and monkey, it would seem probable that it has a general application. On the other hand, it does not follow that all inhibition is of this direct type. On the contrary, Lorente de N6 (7), Gasser (1), and others have advanced strong reasons for the existence of depression conditioned by preceding activity of the cell depressed. To this secondary type of inhibition it might be well to apply Dusser de Barenne's term, "extinction," reserving the name "inhibition" for the primary class of depression with which this paper deals. Such a distinction would place the primary type tentatively in the same category with the analogous case of peripheral inhibition in the autonomic nervous system, in relation to which the word was first employed.

SUMMARY

Inhibition of the ipsilateral flexor reflex by a single afferent volley in a nerve or posterior root of the opposite side has been recorded in terms of the response of interneurons and of muscle in a series of cats, dogs, and monkeys at intervals after transection of the spinal cord ranging from a few minutes to two months.

In the cat crossed inhibition is obtainable with far weaker stimuli than in dog or monkey.

In all three species the depth and duration of crossed inhibition increases progressively with recovery from spinal shock. In all three, the chronic animal in good condition may show a corresponding inhibition of internuncial and reflex response.

* Though the occurrence of occasional interneurons in the anterior horn has been described their number is believed to be too small to alter significantly the internuncial drive.

The briefer the interval between transection and recording and the weaker the inhibitory stimulus, the more evident is a higher resistance to inhibition by interneurons than by motoneurons. In the cat this difference is insignificant and detectable only for a few hours after transection. In the monkey it is frequently extreme for a considerable period and has been demonstrated 59 days after transection.

These results are discussed in relation to the theory of the inhibitory genesis of spinal shock and in relation to the mechanism of inhibition. Evidence is presented for the occurrence of inhibition which is not conditioned by preceding activity of the inhibited neurons.

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CORTICAL ORIGIN AND DISTRIBUTION OF CORPUS CALLOSUM AND ANTERIOR COMMISSURE IN THE MONKEY (*MACACA MULATTA*)*

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THE CORPUS callosum and anterior commissure constitute a fibre system larger than the sum of all systems ascending to and descending from the cerebral hemispheres. Anatomical studies¹ and retrograde³² and Wallerian^{2,24-31,33,34,36} degenerations following lesions of this commissural system have indicated only cortical origins and axonal distributions which were for a large measure cortical and roughly symmetrical.

Little is known of its function. Apart from symptoms referable to lesions of adjacent structures,³⁵ tumors, softenings and surgical sections³⁴ have failed to produce any characteristic disorders except, possibly, impairment of co-ordination of the hemispheres in complicated symbolic activity.³⁵ Even complete transection of the corpus callosum has not invariably prevented the spread of convulsions from one side of the body to the other.³³

Physiological results without electrical records were equally negative.²³ Bishop was probably the first to demonstrate a function referable to the corpus callosum. At the symposium of the American Neurological Association in 1937 he indicated that "when both occipital poles are released from domination by deeper structures, by cutting above the geniculate bodies, the corpus callosum throws these cortical areas of the two hemispheres into exact synchronism." In 1939 Erickson²¹ found that section of the corpus callosum prevented the spread of electrical afterdischarge from one hemisphere to the other. In that same year, by stimulating one hemisphere electrically and recording from the other, Curtis and Bard⁸ mapped the inter-hemispherical communications and, by sectioning the corpus callosum, they proved these to be by callosal fibres.

In 1937, while working on the functional organization of the sensory cortex of the monkey, Dr. Dusser de Barenne and one of the present authors had strychninized 1 sq. mm. of area 4 of one hemisphere and searched the other but found no strychnine spikes. When Curtis published his conclusive study the reason for the failure with strychnine was at once apparent, for the only strychninization had been in an area marked by him as having little or no callosal connection. One cannot hope, by local strychninization to discover any callosal connections which Curtis, by electrical stimulation, has not already disclosed, for strychnine acts only where synapses are present

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† National Research Council Fellow, 1940-1941.

on nerve cells and causes disturbances propagated only in the normal direction,^{9,18} whereas we know no way to prevent electrical stimulation from producing antidromic disturbances and from exciting any axones passing through the stimulated area. It was, therefore, with the hope of clarifying and simplifying Curtis's findings, rather than of amplifying them, that the present study was undertaken.

METHODS

All experiments were performed upon monkeys (*Macaca mulatta*) fully anaesthetized with Dial* (0.45 cc. per kg., $\frac{1}{2}$ intraperitoneal, $\frac{1}{2}$ intramuscular). Both hemispheres were exposed widely, care being taken to preserve the cerebral circulation. For section of the corpus callosum, one side was prepared in advance by section of veins joining the brain to the superior longitudinal sinus. Thirty-six electrodes were placed on one hemisphere and connected, 6 at a time, to 5 channels of a Grass 6-channel inkwriter oscillograph. The arrangement was "linear" i.e., successive channels had one electrode in common. The sixth of successive strychninizations. These were of about 10 sq. mm. so that the major portion of one hemisphere could be strychninized in each animal. When no spikes appeared at any of the 36 electrodes the area symmetrical to the strychninization was examined thoroughly by moving one or more electrodes from place to place over the area in question. Additional electrodes on the hemisphere strychninized were used to identify the site.

RESULTS

When one places on one hemisphere a small piece of filter paper moistened with a saturated solution of strychnine sulphate the surface of the cortex at the site of strychninization rapidly drifts negative and in less than half a minute there appear small fast negative fluctuations.¹⁵ These cannot be detected except at the site of strychninization. Within about one minute a positive deflection precedes each negative fluctuation and a lesser and slower positive deflection follows. This is the fully developed strychnine spike, and it is propagated as a recognizable strychnine spike to all other regions reached by axones from the area strychninized. It was by means of

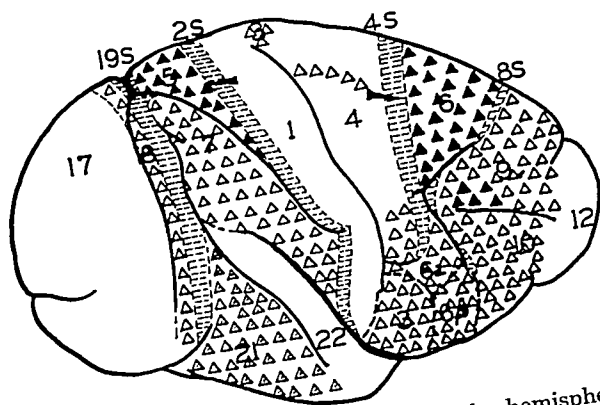


FIG. 1. Map of the convexity of the hemisphere numbered in conformity with Brodmann's nomenclature except for those numbers followed by S to indicate that these areas are identified by their giving suppression of electrical activity. They are indicated by horizontal shading.

- Δ = Projection to contralateral hemisphere at symmetrical focus only.
- \blacktriangle = Projection to contralateral hemisphere at symmetrical and other foci.
- \triangle = Projection to contralateral hemisphere at symmetrical focus only which remains after section of the corpus callosum.

* We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

these strychnine spikes that the functional organization of the sensory and adjacent cortex¹² and of the occipital lobe³ were originally mapped and found to conform in the main to the cytoarchitectonic maps of Brodmann⁴ and the Vogts.^{3,7} The map so produced formed the basis for the present experiments in determining what physiologically unique area was strychninized on one hemisphere or recorded on the other. Figure 1 shows such a map of the convexity of the hemisphere on which are plotted schematically the results of the present investigation.

In these experiments many minute, rounded or slightly belated disturbances of the opposite hemisphere were encountered. These have been consistently excluded in making Fig. 1, for it was feared that these might be electrical spread from structures other than those of the cortex subjacent to the electrodes (e.g., fibre tract) or even post-synaptic disturbances. Thus, this diagram represents only the origin of indubitable commissural projections which pass from the cortex of the convexity of one hemisphere to the cortex of the convexity of the other without relay.

None of the suppressor areas, 8s, 4s, 2s or 19s, gives rise to contralateral strychnine spikes, nor do areas 1, 12, 17 or 22. Area 9, except for a portion above the sulcus principalis, area 10, area 6a and b (face), area 4 (trunk, neck and face only), area 7, area 18 and area 21 give rise to contralateral disturbances restricted to foci symmetrical to the focus strychninized. Whereas part of area 9, above the sulcus principalis, area 6 (leg and arm) and area 5 (leg and arm) give rise to disturbances which are distributed to large areas of the opposite hemisphere. In fact, leg 6, arm 6, leg 5 and arm 5 give rise to disturbances of both pre- and postcentral sensory areas of the opposite hemisphere.

With one exception, section of the corpus callosum prevents the propagation of the strychnine spikes to the opposite hemisphere. Partial section pre-

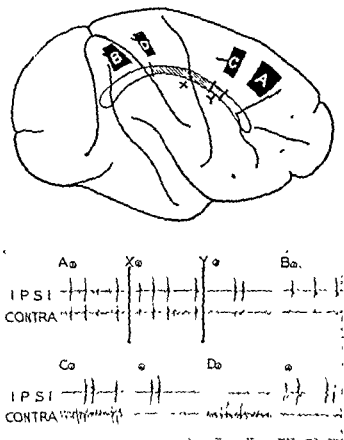


FIG. 2. Feb. 28, 1941. *Macaca mulatta*. Dial. Diagram of the convexity of the right hemisphere indicating sites of strychninization. On this is projected the corpus callosum indicating two partial sections thereof, X and Y. All records were made with bipolar pickup electrodes at the site of strychninization (ipsi) and on the contralateral symmetrical point (contra). Record A① was made before section X; Record A②, after section X but before section Y; Record A③, after section Y. Records A④ and B① show the minute residual disturbances of the contralateral hemisphere. Records C② and D② show suppression of the electrical activity of the contralateral hemisphere.

vents their propagation from part of the hemisphere. Figure 2 shows diagrammatically such an experiment. Following a large strychninization, A, of area 6 of one hemisphere which was projected to the opposite hemisphere (Record A1) the corpus callosum was partially sectioned (cf. X in the diagram and at time X in the record). The strychnine spikes came through to the opposite hemisphere unaltered (Record A2). The section was then extended (cf. Y in the diagram and at time Y in the record). Then there remained only very small spikes (Record A3). Thereafter several strychninizations occipital to strychninization A were performed on areas known to have callosal projections, but no spikes appeared on the opposite hemisphere

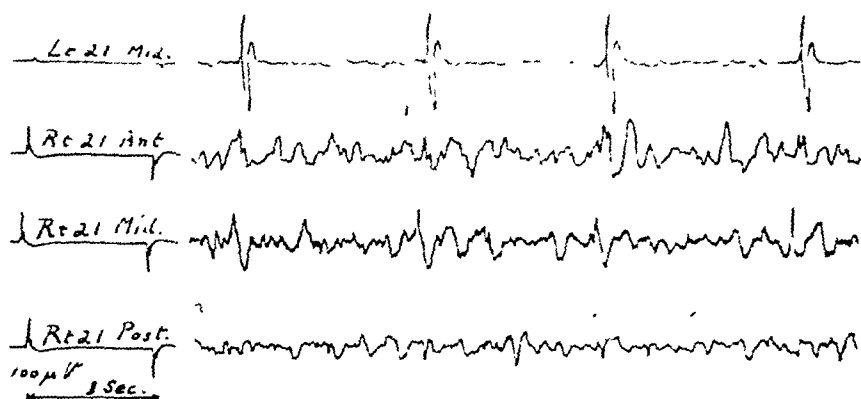


FIG. 3. May 28, 1941. *Macaca mulatta*. Dial. Strychninization of entire left second temporal convolution, area 22, twenty minutes after complete transection of corpus callosum. Note strychnine spikes are largest in the record of the middle of the right second temporal convolution.

until strychnine was placed as far occipitally as B, which is in arm 7, when minute disturbances (Record B1) again appeared on the opposite hemisphere. Briefly, the section X+Y had interrupted almost all transmission via the corpus callosum from one sensory cortex to the other.

The exception mentioned above occurs in the case of area 21, the mid-temporal convolution. Strychninization in each part of this area resulted in typical spikes sharply restricted to the symmetrical focus of the opposite hemisphere. Section of the corpus callosum failed to prevent their occurrence. Figure 3 shows these spikes, as disclosed by the roving electrodes at the site and as recorded from the contralateral hemisphere. This strychninization covered practically the entire convolution and hence it is probably significant that the spikes on the contralateral hemisphere are larger and more constant in the middle third of the convolution than in either the anterior or posterior third. In this experiment the anterior commissure was intact.

In a second experiment it also was divided and thereafter no strychnine spikes were transmitted to the opposite hemisphere. Moreover, following section of the entire corpus callosum, electrical afterdischarge following

stimulation of area 21 on one hemisphere spread to the same area of the other hemisphere, and this spread was also prevented by section of the anterior commissure.

Suppression of the electrical activity of one hemisphere by strychninization of 8s, 4s, 2s and 19s of the other hemisphere has also been observed. This suppression has been obtained after section of the corpus callosum. Records C1, C2, D1 and D2 of Fig. 2 instance such suppressions of the activity of the contralateral hemisphere after strychninizations of 4s and 2s which lie within an area all of whose callosal connections had already been severed.

DISCUSSION

The known anatomy and physiology of the corpus callosum and anterior commissure and the known properties of the strychnine spike would lead one to expect at least all the positive findings of direct interhemispherical connection here presented. In fact, these constitute, so far as the callosal system is concerned, a partial confirmation of those of Curtis,⁵ from which they differ only privatively. Due to the relatively large strychninizations and the large number of foci recorded following each strychninization it is not likely that the differences are due to oversight. It is possible that potentials which were regarded as of questionable significance, and so excluded from Fig. 1, would have been included by Curtis as small disturbances, but certainly the greatest reason for the differences must be sought in the dissimilarity of stimulation. Such positive findings of commissural connections as are obtained by its use indicate cell bodies which are situated in the area strychninized and give rise to axones extending to the site of appearance of the strychnine spikes. From the voltage of these spikes it is highly probable that many axones must participate, although it is impossible to say how many. Systematic exclusion of small and questionable disturbances may well have prevented indication of callosal connections which were comparatively scattered and not numerous. Thus Fig. 1 is in no sense a complete chart of interhemispherical connections but merely a diagram of the lateral aspect of one hemisphere on which are indicated those of its areas which give rise to numerous callosal axones ending in the cortex of the convexity of the opposite hemisphere. As less than one-sixth of the total cortex of one hemisphere is on the surface of the convexity Fig. 1 cannot represent more than one-sixth and may represent as little as one thirty-sixth of the total area giving axones to the commissural system.

The primary projection areas of vision and of somatic sensation are markedly deficient in callosal projections whereas so-called associational areas have them in abundance. Similarly when one looks at the primary motor area, 4, one finds these callosal connections restricted to areas controlling parts of the soma which are most frequently used symmetrically, and lacking from those representing the extremities which are moved independently. On the motor side of the sulcus centralis the greatest callosal projection arises

from areas leg 6 and arm 6 whose stimulation yields more complex movements.

That section of the corpus callosum failed to prevent firing of one area 21 by the other, and that this firing occurred without time for relay in other

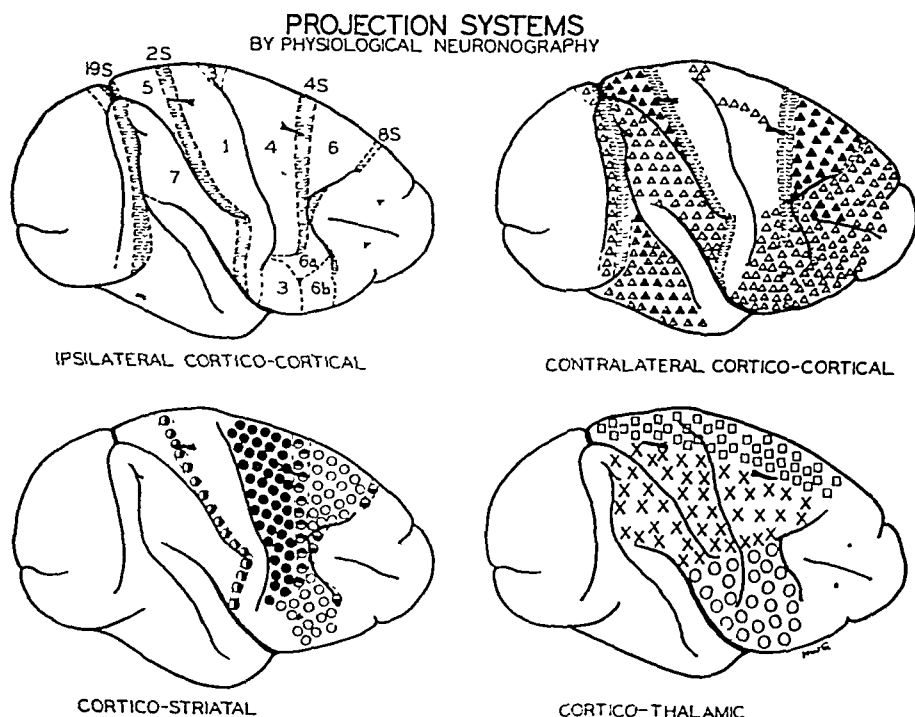


FIG. 4. Numbers indicate portions of the sensory and adjacent cortex distinguished on the basis of their firing into other areas or being fired into from other areas of the same hemisphere except those numbers followed by S which yield suppression of electrical activity of the cortex. Horizontal shading indicates areas identified by suppression.

- △ = Projection to contralateral hemisphere at symmetrical focus only.
- ▲ = Projection to contralateral hemisphere at symmetrical and other foci.
- ◻ = Projection to contralateral hemisphere at symmetrical focus only which remains after section of the corpus callosum.
- = Projection from area 2s to nucleus caudatus.
- = Projection to putamen.
- ◐ = Projection from area 4s to nucleus caudatus.
- = Projection to putamen and to external segment of globus pallidus.
- ◑ = Projection from area 8s to nucleus caudatus.
- = Projection to leg nuclei of the thalamus.
- × = Projection to arm nuclei of the thalamus.
- = Projection to face nuclei of the thalamus.

structures indicates that cells situated in area 21 have axones passing to the other hemisphere by a bridge other than the corpus callosum. Anatomical considerations by exclusion strongly indicate that this must be by way of the anterior commissure—a conclusion confirmed by failure to cross following section of the anterior commissure.

Since it is clear from previous work of this laboratory,¹⁴ and from Erickson's study,²¹ that electrical after discharge (and therefore the central disturbance in clonic seizures) spreads by neuronal paths it is to be expected that section of the corpus callosum alone would prevent the spread of many seizures from one side to the other. The remaining connection of the areas 21, by way of the anterior commissure, should suffice for the crossing if the disturbance spread to the temporal lobe on the first side. Erickson²¹ had not investigated these regions from, and to which crossing occurs via the anterior commissure. As shown by the present findings of spread of after discharge, these may play a significant role in those patients of whom Van Wagenen²³ reports that they had seizures recurring bilaterally after section of the corpus callosum. Thus, apart from its surgical implication, this finding concerning the anterior commissure is significant in explaining the apparent contradiction between Erickson's conclusions and Van Wagenen's reports.

Two new findings with respect to the suppressor areas—(i) that suppression of electrical activity is bilateral, (ii) that it remains bilateral after section of the corpus callosum—are in harmony with conclusions from previous experiments.^{13,16} Since the path responsible for suppression of electrical activity is known to be subcortical and the effect is bilateral, and since suppression of motor response^{17,19} to cortical stimulation is necessarily subcortical, and bilateral, the third finding is not surprising—namely, that the areas (8s, 4s, 2s and 19s) responsible for these suppressions give rise to no demonstrable callosal projection.

Curtis has already called attention to the failure of the callosal system to correspond to cytoarchitectonic or somatotopic subdivision of the cortex.⁵ Figure 4 amplifies this point by contrasting four maps of the convexity of the hemisphere as so far revealed by physiological neuronography. The upper left shows how that cortex can be divided on the basis of corticocortical connections.^{3,12,14,20,22} The suppressor areas (8s, 4s, 2s and 19s) lack such connections. The upper right shows the principal origins of the corpus callosum and anterior commissure. Again the suppressor areas lack those connections. The lower left shows the areas separated on the basis of their projection to basal ganglia.^{13,16} Here all half-filled circles indicate connections to the nucleus caudatus. All come from the suppressor areas, and stimulation of the nucleus caudatus yields suppression. The lower right indicates origins of the essentially somatotopic projections to the leg, arm and face nuclei of the thalamus.^{10,11} Thus, this figure reveals the unique origin of each of these systems. The corpus callosum and anterior commissure are no exceptions.

CONCLUSIONS

Based on local strychninization of one hemisphere and electrical records of the other, *i.e.*, by physiological neuronography, a new map of the convexity of the cerebral hemisphere of *Macaca mulatta* has been prepared to show the origins of the corpus callosum and anterior commissure and to indicate (i) those origins whose interhemispherical projections are dispersed to

many areas of the convexity of the other hemisphere, and (ii) those whose projections are restricted to symmetrical foci.

The former spring from a part of area 9 above the sulcus principalis and from areas 5 and 6 of the leg- and arm-subdivisions. The latter have been found to arise from many parts of the frontal pole, from face 6, from the trunk and face portions of area 4 and from areas 18 and 21.

The last of these restrictedly symmetrical projections, *i.e.*, that between the areas 21, is unique, for it alone remains after section of the corpus callosum, provided the anterior commissure is intact.

Suppression of electrical activity of the cortex by strychninization of 8s 4s, 2s or 19s has been found to be always bilateral, even after section of the corpus callosum to which these areas contribute few or no axones.

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CORTICAL ORIGIN AND DISTRIBUTION OF CORPUS CALLOSUM AND ANTERIOR COMMISSURE IN CHIMPANZEE (*PAN SATYRUS*)*

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THE interhemispherical communications of the sensory cortex have been investigated in the same manner and often in the same animals as the functional organization of the hemispheres separately,^{8,11} i.e., by the local application of strychnine and the recording of the resultant alteration of electrical activity. Because those studies of the sensory cortex had shown that the investigation had to be extended not only to adjacent¹ but even to remote² parts of the same hemisphere these were also strychninized and recorded on both hemispheres. Finally, in view of what had already been found concerning the temporal lobe and the anterior commissure in the monkey,¹⁵ this region was also investigated before and after section of the corpus callosum. Thus, these experiments have extended step by step to include the entire convexity of the hemispheres. Since these findings all pertain to the commissural system which subserves a relatively distinct type of functional organization, they are presented separately in this article.

METHODS

The experiments were performed upon six chimpanzees (*Pan satyrus*) fully anaesthetized with Dial§ (0.35 cc. per kg., $\frac{1}{2}$ intraperitoneal, $\frac{1}{2}$ intramuscular). Both hemispheres were exposed by turning down large bone flaps. For experiments on the temporal region and on the temporo-parietal angle the zygomatic arches and the temporal muscles were removed and the temporal bones cut back as far as vascular structures permitted. For investigation of the orbital surface of the frontal lobe the orbit was exenterated and its bony roof removed. Previous to sections of the corpus callosum and anterior commissure the sagittal arch between the bone flaps was removed and the vascular attachments of one hemisphere to the longitudinal sinus and the falx were cauterized and severed. Sections of the commissural systems were made with a blunt instrument and no bleeding was encountered. By so tipping the board to which the animal was attached that the heart was level with the head, by carefully preserving all vessels that did not have to be severed, and by avoiding trauma to the arachnoid membrane so that the cerebrospinal fluid did not escape, the cortex was kept in good condition without irrigation for an exposure lasting as long as three days.

Twelve electrodes were applied to the hemisphere to be strychninized and thirty-six to the other. By appropriate switching, six of these at a time were connected in the "linear" hook-up to five channels of a 6-channel Grass inkwriting oscillograph. The sixth channel was used for roving electrodes at the site of strychninization. In several experiments strychninizations were performed on the hemisphere with the thirty-six electrodes to work out

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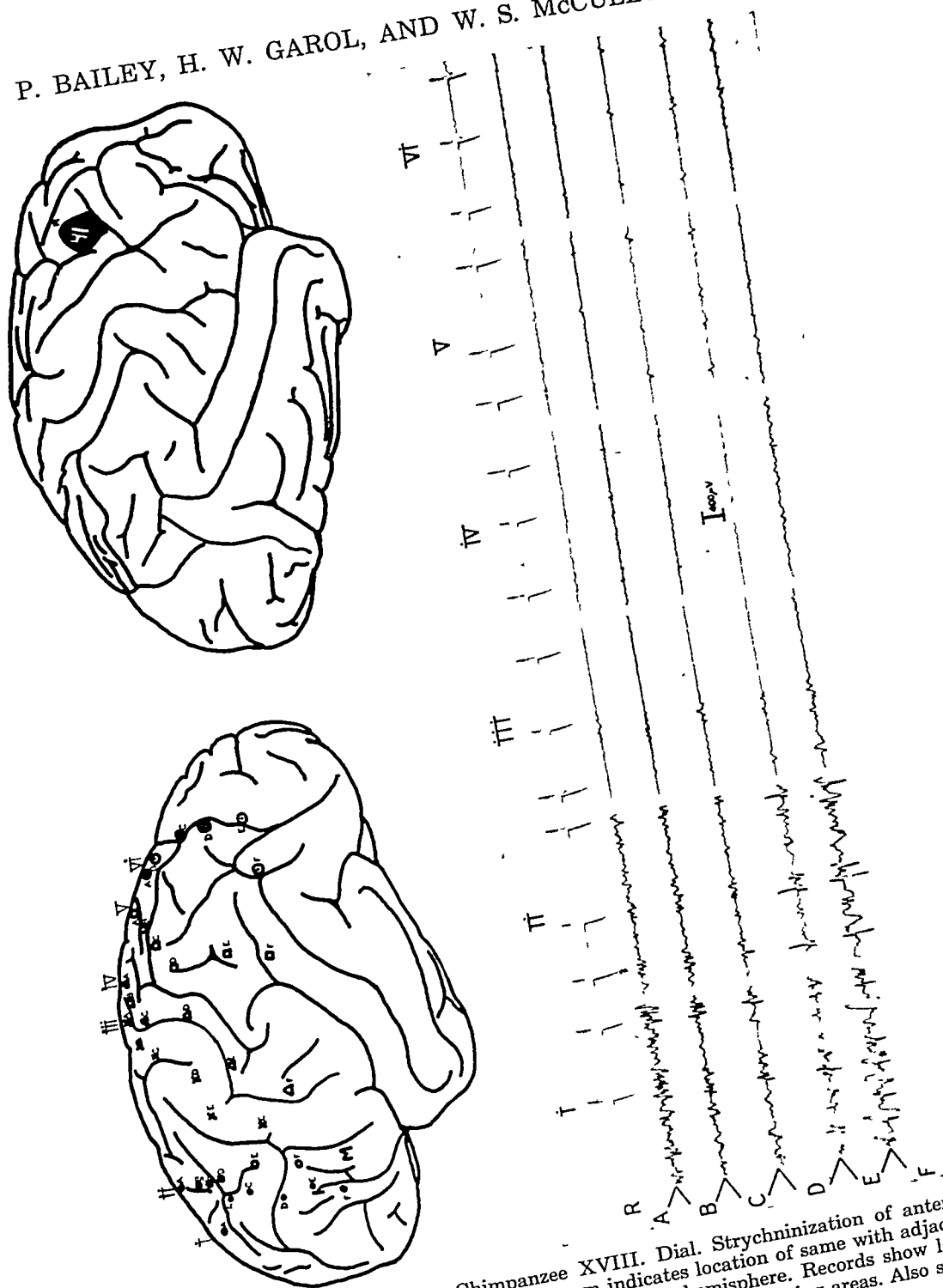


FIG. 2. May 27-28, 1941. Chimpanzee XVIII. Dial. Strychninization of anterior margin of Band II. Right hemisphere. Diagram indicates location of same with adjacent rover electrode and location of electrodes on opposite hemisphere. Records show large strychnine spikes at symmetrical focus and smaller ones in neighboring areas. Also small disturbances in posterior part of sensory cortex and in vicinity of sulcus lunatus.

in the figure, were all strychninized but gave rise to no certain or recognizable contralateral strychnine spikes.

Obviously, this figure fails to indicate the termination of commissural systems which fire other areas in addition to the symmetrical focus. Both such areas in the postcentral cortex cross only into the postcentral cortex. The upper, in the superior parietal lobule, is distributed to Band IX, leg, trunk, arm and face; the lower, in the lower end of the postcentral convolution, into Bands VIII and IX, face and arm.

The widest contralateral distribution is from Band II. All parts of this band fire to symmetrical foci and all solid triangles are at places which fired to other subdivisions, leg, arm or face, of Band II contralaterally. Moreover, strychninizations in each of these subdivisions of Band II produced contralateral firing of more than one subdivision of Band IV and sometimes, though only questionably, of Band V. All subdivisions of Band II have also fired into some postcentral portion of the opposite sensory cortex. This firing was most limited from face II, which fired face VI and from trunk II, which fired only one part of arm X; next, from leg II which fired into the gyrus above the sulcus interparietalis, presumably trunk VIII and IX, as well as into leg and arm IV; and, least restricted, from arm II, which fired into all of the areas affected by leg II and also into the anterior lip of the sulcus lunatus. Figure 2 exemplifies this finding.

With one exception, all firing of the hemisphere contralateral to the strychninization ceased when the corpus callosum was divided. This exception was found in the temporal lobes where strychninization of the middle and posterior thirds of the second temporal convolution still fired symmetrically after, although at a lower voltage than before, the section (see Fig. 1).

Following strychninization of each of the suppressor bands (see Fig. 1) bilateral suppression of electrical activity of the cortex has been observed. The activity diminished in corresponding areas at approximately the same time on the two sides but, as the electrodes were not arranged for simultaneous records, with the one exception of the rover, discrepancies of less than one minute could not have been detected (see Fig. 3). These suppressions remained bilateral after section of the corpus callosum.

DISCUSSION

The topography of the chimpanzee's cortex is very variable and the present report is based on only 6 chimpanzees—a total of only 223 strychninizations. Moreover, the cortex of the chimpanzee is so large and so functionally differentiated, and the area which shows a contralateral strychnine spike can be so small even when the strychninized area is relatively large, that 36 stationary electrodes are far too few for an exhaustive survey of even a restricted area such as the frontal lobe or the temporal lobe or the sensory cortex. Although these electrodes were supplemented by frequent attempts to locate symmetrical disturbances by moving electrodes many disturbances, particularly if they involved only small, unsymmetrical areas, may have been

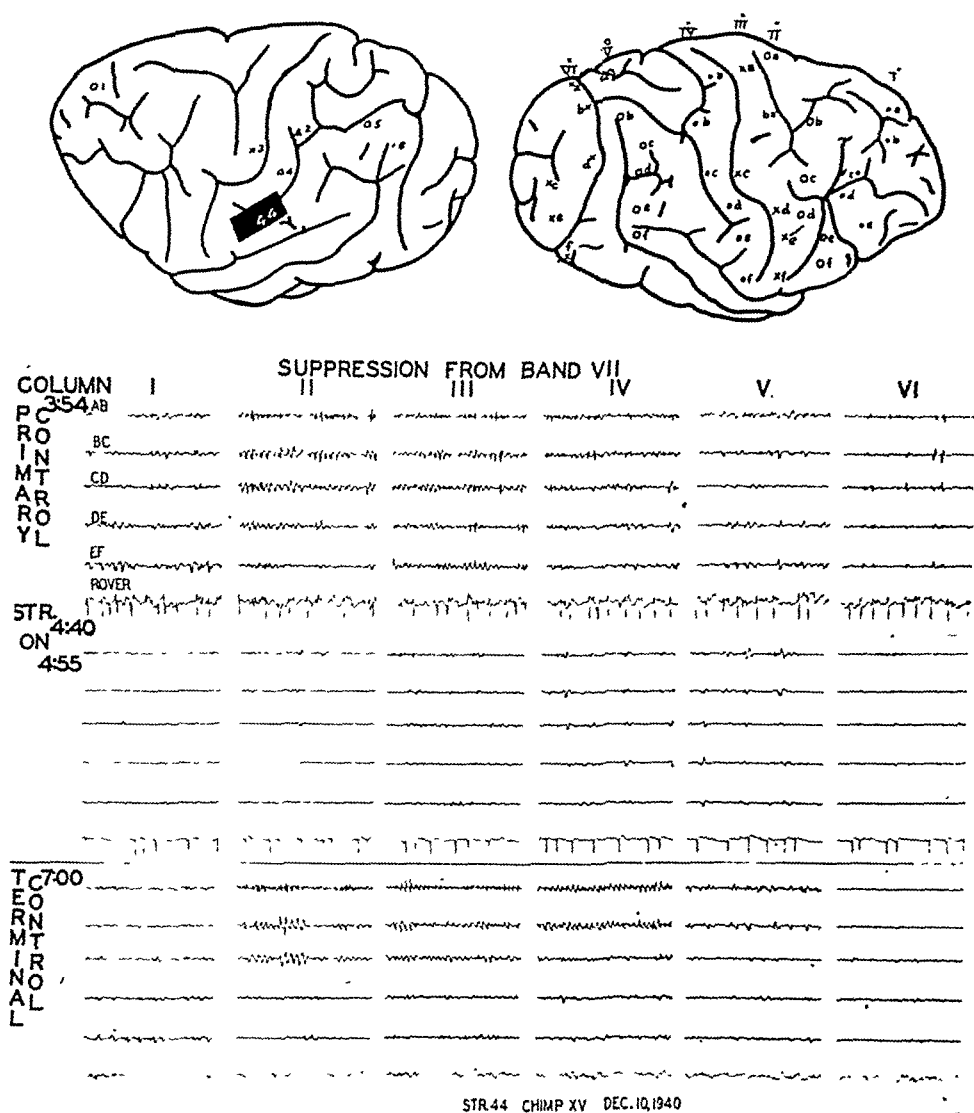


FIG. 3. Dec. 10, 1940, Chimpanzee XV. Dial. Strychninization number 44. Diagrams of location of strychnine and electrodes in the most prolonged suppression of the contralateral hemisphere as yet seen in the chimpanzee. After primary control rover moved from site of previous strychninization to that of strychnine 44. Note—activity not fully returned in 2 hours 20 minutes.

missed. For this reason, as well as for those stated in the articles on the commissural systems of the cat¹⁵ and the monkey,¹⁶ the map of the lateral aspect of the chimpanzee's cortex showing the origin of callosal systems must be regarded as incomplete. It indicates only the chief origins of the callosal system which lie on the convexity of the hemisphere and terminate in a relatively concentrated manner upon the convexity of the opposite hemisphere.

During these studies it has become increasingly apparent that Curtis^{4,5,6} and Curtis and Bard⁷ were correct in their description of the extremely circumscribed, exactly symmetrical, type of contralateral representation. This functional commissural connection is far more precise and restricted than is suggested by the term "homeotopic" used to describe the corresponding anatomical connections. Moreover, in every case we have confirmed their finding of symmetrical firing even when the origin excited fired widely distributed areas of the contralateral hemisphere. This must mean anatomically that with every heterotopic is intimately associated a homeotopic callosal connection. The association seems to be due to the fine structure of the brain rather than to excitation of adjacent but dissimilar areas, for it was found by Curtis with electrical stimulation near threshold strength, and by the present authors with the smallest strychninization.

This is only one of several noteworthy points of similarity in these studies of cat, monkey and chimpanzee. Several need only be mentioned, such as the failure to discover any commissural connections from any suppressor areas, from those parts of area 4 which control parts of the body used most independently (i.e., leg 4 and arm 4) and from area 17. While there is no satisfactory chart of the cytoarchitecture of the chimpanzee, if one homologizes on the basis of functional organization,^{1,9-14} and uses Brodmann's numbers³ throughout it is possible to state some of the remaining similarities briefly. Area 18 and part of area 3 and at least some part of the face-subdivision of area 4 yield in each animal restrictedly symmetrical disturbances which are entirely mediated by the corpus callosum, whereas area 21, which has such a projection, utilizes, in part at least, the anterior commissure. Finally, while the widespread disturbances are encountered only from restricted portions of the cortex which are neither "motor" nor "sensory"¹⁷ in the most restricted sense, these origins do not lie in the newer associative areas nor enlarge as these enlarge. Unfortunately area 6 was not investigated in the cat, but in both the monkey and the chimpanzee it is the source of the widest callosally mediated disturbances. Similarly area 5 gives rise to widespread contralateral disturbances, although not to as remote areas. In the cat, the part of area 7 adjacent to area 5 has similar properties. In the monkey this area has only origins of symmetrical disturbances, and in the chimpanzee in which "area 7" has either further differentiated or been invaded by newer association areas, only part of the region has origins of the restrictedly symmetrical type, and the rest, none. This may be a development comparable to the differentiation in the face region in front of the Rolandic fissure where, in the chimpanzee, the upper half of the face region no longer gives rise to any discoverable commissural system, although the neck region (above) and the lower face region (below) have such connections.

Three other small areas gave dissimilar findings in monkey and chimpanzee but the authors have no way of knowing whether these are due to areas being in the depths of a sulcus in one animal and on the convexity of a convolution in the other, or to changes comparable to those in "area 7".

The first is found above the sulcus principalis in area 9 of the monkey, and the other two appear in the chimpanzee, one just in front of the lower part of Band IV in the face-subdivision, and the other just above the Sylvian fissure below and behind the end of the sulcus interparietalis. In this connection it should be added that the longest projection from the frontal margin of the arm-subdivision of area 6 (Band II) to the contralateral area 18, was found in the chimpanzee, but diligent search failed to reveal it in the monkey, in whose cortex arm 6, where it adjoins area 8, dips into the sulcus arcuatus. Thus these discrepancies may merely be examples of the incompleteness of our knowledge, due to failure to excite the origin or record the termination of the part of the commissural system under investigation.

It must, finally, be emphasized that these studies are complementary to those on the functional organization of one hemisphere and no attempt has been made to detect anything except origins and terminations which are cortical.¹⁸

CONCLUSIONS

By local strychninizations of one hemisphere and recording electrical activity of both, a new schematic map of the convexity of the hemisphere of *Pan satyrus* has been made to indicate the principal origins of the corpus callosum and anterior commissure. It shows (i) those origins of commissural fibers which project to many areas on the convexity of the other hemisphere and (ii) those which project only to symmetrical foci. The former arise from all subdivisions of Band II and from two small areas, one above the superior parietal sulcus and the other below and behind the inferior extremity of the interparietal sulcus. The latter come from many regions of the frontal pole, from scattered parts of Band II, from the trunk, neck and lower face regions of Bands IV and V, from Band IX and from a region adjacent to the sulcus lunatus (presumably area 18) and from the central and posterior thirds of the second temporal convolution (probably area 21).

The last of these origins is unique in that strychnine spikes continue to be propagated (although at reduced amplitude) to the symmetrical focus following section of the corpus callosum and, hence, it must give rise to axones which are to be sought in the anterior commissure.

Suppression of electrical activity of the cortex by strychninization of Band I, III, VII or XI has been found to be bilateral even after section of the corpus callosum to which these bands contribute either no axones or too few to yield a recognizable strychnine spike.

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POTASSIUM AND WATER CHANGES IN EXCISED NERVE ON STIMULATION

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FENN (1940) has discussed the now generally recognized loss of potassium by glands, muscles and nerves, during activity. In unmyelinated crab nerves a clear loss has been shown by Cowan (1934) and Young (1938). They produced changes of 6-7 per cent after 5-15 minutes of electrical stimulation. On the other hand, Fenn (1934, 1938) found no significant changes after stimulation of the myelinated nerves of frogs and cats. The question is still open therefore whether myelinated nerves are an exception to the general rule or whether they can be made to lose potassium under proper conditions.

The above mentioned investigators generally employed stimulation periods of 30 min. at most, and did not test the response of the nerves directly. This paper shows that excitation of myelinated frog nerve must be continued for more than 30 min. before any change is evident. Fenn (1934) did apply tetanizing currents to frog nerves for as much as 6 hours, with a consequent loss of K, but his experiments were too few to be significant.

In our work, the stimulation times ranged from 13 min. to 3.5 hr. The response of the nerves was followed by measuring the height of the diphasic action potentials on a cathode ray tube.

METHOD

The sciatic nerves of two frogs were used for each experiment. After equilibration for at least two hours in Ringer's solution† at 5°C., one pair (S pair) was placed in 1.2 cc. of the solution in the stimulating chamber (S chamber). This consisted of a narrow groove cut into a paraffin block, covered with a glass plate. At one end of the groove, a closely-spaced pair of platinized electrodes was used for stimulation, and a similar pair at the opposite end was used for recording. The electrodes were separated from the middle part of the groove by partitions, and the salt solution was confined to this central part. Air was bubbled through the bath, after first passing through an external reservoir of the same solution.

The main body of the nerve-pair was immersed in the central part of the groove; the nerve ends were drawn out of the bath, across the partitions, and placed on the electrodes. Thus the electrodes were kept in air, and the stimuli did not pass through the fluid and the whole nerve trunk, as was the case in Cowan's procedure (1934). The stimuli were slightly supramaximal thyatron-controlled condenser discharges, adjusted to 60 per sec. in all trials.

After stimulation, the nerve ends which had projected beyond the bath were cut off and the remainder of the trunk was analyzed for potassium. The analyses were made by

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‡ Composition, in gr. per liter solution: NaCl 6.5, KCl 0.14, CaCl₂ 0.12, NaHCO₃ 0.20, NaH₂PO₄ 0.01. Total K, including impurities, = 0.074 mg/cc. solution.

W.S.W. using a modified Shohl and Bennett method (Wilde, 1939). Amounts of K as small as 0.035 mg. were measured with an average variation of ± 1.5 per cent.

A separate chamber (C chamber), identical with the S chamber, was provided for the control nerves (C nerves). For each experiment, a pair of C nerves was run simultaneously with the S nerves, though only the latter were stimulated. The response of the controls in groups A and B was measured by giving them a few test shocks at the beginning of a run, and again at the end. The potentials of these controls showed no deterioration during any of the runs.

RESULTS AND DISCUSSION

Changes during activity. Table 1 consists of the original data, separated into 3 parts according to the duration of stimulation. No nerves were stimulated in the third group. These tests simply provided an additional check on the method of selection, which was as follows: the right sciatic of one frog and the left sciatic of a second frog were used for stimulation. The remaining two nerves were used as controls.

In the last 7 experiments of Table 1 this procedure was followed as usual, except that the nerves in the S chamber were not stimulated. There was no significant difference between these and the C nerves in regard to either K or water (group C, Table 2). There was still no definite change in group B, activated for 13–30 min., but group A, activated for 60–210 min., showed a loss of K without any corresponding shift in water (unaltered dry weight).

In two of the tests (12, 14) the movements of K were followed by analysis of the bath as well as the nerves. In each case the rise of K in the fluid indicated a loss of 18–20 mg. per cent per hr. by the stimulated nerves. This agreed with the values of 14–20 mg. per cent per hr. shown by direct analysis of the nerves. In fact, all the trials in the first group (60–210 min.) showed losses during activity within a range of 10–20 mg. per cent per hour, with the exception of the two showing gains (no. 3, 13).

Cowan's results are in sharp contrast (1934). He reported a leak of about 180 mg. per cent per hr. from the unmyelinated nerves of crabs as a result of stimulation. However, stimulation was continued for only 10 min. It would be of interest to see how long the rate of loss could be maintained.

The smaller change in frog nerve may be due to the myelin sheath. The sheath seems to be impermeable to electrolytes, considering the high inter-nodal resistance recorded by Tasaki (1939). The high conductivity at the nodes suggests that this might be the only place where K can escape. Erlanger and Blair (1938) offer additional evidence in support of this view. They state that isotonic glucose blocks frog nerve by reducing axon excitability at the nodes, where K presumably leaks out.

Changes during soaking. Table 3 shows the difference between short and long soaking on the K and water contents of the C nerves. Prolonged immersion caused a definite drop in the dry weight fraction, indicating a gain of 9.5 per cent in the amount of water associated with each 100 gm. of dry weight.

Prolonged immersion also resulted in a fall in concentration of K in nerve water. However, the concentration in dry nerve was not significantly

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Table 1. Analytical data

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Table 1. Analytical data

Exp.	Mg. wet nerve		Per cent dry weight		Mg. per cent K in wet nerve		ΔK wet per cent	Stim. min.	Final potential per cent	Temp. °C.	Soaking hr.
	C	S	C	S	C	S					
Group A—Long Stimulation											
1	31.7	32.4	26.8	26.5	227	176	-22.5	210	50	25	5
6	20.9	23.1	23.9	29	187	156	-16.6	184	25	15	5
12	24.6	26.4	24.8	24.6	219	170	-22.4	154	40	15	10
14	38.8	41.6	27.1	26.4	183	151	-17.5	140	71	23	100
5	20.2	19.1	32.2	44	307	272	-11.4	139	33	15	5
4	37.3	37.4	28.4	28.6	236	208	-11.9	110	46	25	20
13	45.7	41.9	26	24.8	173	184	+ 6.4	90	34	16	10
2	35.6	34.4	26.4	30.5	222	209	- 5.9	85	77	26	2
3	44.4	46.6	25.2	26	180	184	+ 2.2	75	50	25	20
11	—	25.4	—	25.6	—	197	(-27.5)	60	47	16	40
Group B—Short Stimulation											
15	34.7	36	25.4	27.2	210	189	-10	30	90	29	25
16	40.3	38	28	28.7	213	245	+15	30	93	29	10
17	34.5	33.7	28.1	28.8	203	234	+15.3	30	90	29	10
18	36.6	37	27.1	27	218	221	+ 1.4	30	83	29	2
19	35	32.1	24	26.2	214	209	- 2.3	30	82	29	20
20	34.5	35.2	29	29	223	210	- 5.8	30	87	29	20
21	30.4	29.9	28.3	24.8	207	184	-11.1	30	88	29	30
22	30.3	31.3	27	27.2	214	198	- 7.5	30	82	29	25
23	30.7	30.5	27.1	27.2	223	213	- 9	30	91	29	25
24	28.7	29.3	25.8	24.9	233	211	- 5.4	30	96	29	5
10	24.8	24.7	27.8	27.5	238	231	- 2.9	30	63	16	2
7	39	38	28	28.7	208	210	+ 1	13	57	14	2
9	48.5	50.3	25.4	25.7	194	199	+ 2.6	13	69	16	15
Group C—No Stimulation											
8	37.7	36.6	25.5	26.2	228	221	- 3.1	—	—	—	28
25	24.2	22.4	24.8	26.3	206	227	+10.2	—	—	—	15
26	42.2	44.8	26.3	27.7	220	214	- 2.7	—	—	—	2
27	28.9	27.7	30.4	28.2	214	231	+ 7.9	—	—	—	2
28	36.4	36.2	24.7	22.7	198	204	+ 3	—	—	—	30
29	31	30.7	23.2	24.1	200	202	+ 1	—	—	—	30
30	47.4	44.2	22.2	23.5	192	203	+ 5.7	—	—	—	20

S—stimulated nerves. The final column refers to the soaking of
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C—control nerves. S—stimulated nerves. The final column refers to the soaking of control nerves only, during equilibration and control periods combined. In exp. 11 the control nerve was lost; the change in potassium was determined by analysis of the Ringer bath; in all other instances, $\Delta K = (S - C/C) \times 100$. The final group of 7 experiments provides a check on the method of selection; the nerves in the "S" columns were not actually stimulated. The final potential was measured in per cent of the initial value obtained at the onset of stimulation; figures are given for the "S" nerves only.

altered. The loss of K calculated on the basis of nerve water must then be only an apparent one. The real change is evidently a gain of water, resulting in a decrease of the dry weight fraction.

Although Table 3 shows no further loss of K after 2-5 hr. of soaking there is evidence of a small loss from resting nerve during this shorter period, as shown by an increase in K concentration in the bath in 3 cases (11, 12, 14) in which analyses were made. The losses recorded per hour were 1 mg. per cent for 100 hr. (no. 14); 2 mg. per cent for 40 hr. (no. 11); and 4 mg. per cent for 10 hr. (no. 12). Presumably most of this loss occurred in the first

Table 2. Average per cent changes due to stimulation

Group	No. of cases	Stim. min.	K in wet nerve	K in dry nerve	K in nerve water	Nerve dry weight
A	8	60-210	-11.0 ± 2.6	-13.7 ± 3.2	-9.6 ± 2.7	+4. ± 2.2
B	13	13-30	-1.4 ± 1.6	-1.2 ± 1.7	-1.5 ± 1.8	-1 ± 1.3
C	7	0	+3.2 ± 1.3	+2.4 ± 2.3	+3.6 ± 1.3	+1.2 ± 1.6

The values given are the means plus or minus the probable error of the mean.

$$\Sigma \left(\frac{S-C}{C} \times 100 \right)$$

$$\text{mean} = \frac{\quad}{n}; \quad \text{P.E.} = \frac{.6745}{\sqrt{n(n-1)}} \sqrt{\Sigma d^2}$$

where d = deviation from mean. Exp. 5 and 11 are not included in group A, in no. 5 the dry weight values were abnormally high, and in no. 11 the C nerve was lost.

Table 3. Changes in control nerves due to soaking

Hrs.	No. of cases	Mg. per cent K in wet nerve	Mg. per cent K in dry nerve	Mg. per cent in nerve water	Per cent dry weight	Cc. H ₂ O per 100 gm. dry
2-5	10	217.8 ± 3	801.1 ± 10.5	299.9 ± 4.8	27.3 ± .4	268.3 ± 4.9
25-100	10	207.5 ± 3	816.7 ± 14.4	279. ± 4.7	25.5 ± .4	293.8 ± 6.2
Av. per cent diff.		4.7 ± 1.9	1.9 ± 2.2	7. ± 2.2	6.6 ± 1.8	9.5 ± 2.9

The values in the first two lines are means ± P.E. and in the third line the "Av. per cent diff." is the percentage difference of the above two means ± P.E. of the difference (P.E. diff. = $\sqrt{(\text{P.E.}_1)^2 + (\text{P.E.}_2)^2}$). Exp. 5 and 11 are excluded from this table because of abnormal dry weights in the former, and loss of the C nerve in the latter. Exp. 26-29 provide 2 C nerves each.

2-5 hours at a calculated rate of 8-20 mg. per cent per hr., although the gain of water continued for a longer period. Fenn *et al.* (1934) reported that frog nerves soaked in a solution similar to ours and containing 8 mg. per cent of K lost 2-3 mg. per cent per hr. for 16 hr. which is equivalent to 8 mg. per cent per hr. for 5 hr. Cowan's (1934) estimate for unmyelinated crab nerves is 6-12 mg. per cent per hr. for 1 hr.

There is some divergence between the average K values of frog nerve as reported by us and by Fenn *et al.* Their figures were 188 ± 17 mg. per cent in spring frogs, 118 ± 10 in winter frogs. We found 211 ± 2 in both

classes based on 35 controls, or 218 ± 3 in the 10 controls with the least soaking. The lowest value for any single control was 173 mg. per cent.

Action potential. On the average the figures of Table 1 show that the action potential decreased to 47 per cent of its initial value after long stimulation (Group A) and only to 82 per cent after short stimulation (Group B). This decrease was presumably due to the stimulation rather than to the soaking, since the potentials of the C nerves of the two groups did not decline during the experiments. The change in the S nerves may have been due to the loss of potassium which was measurable only in group A where the fall of action potential was marked. However, within group A there was no evident correlation between low action potentials and large losses of K. Low potentials rather go hand in hand with low temperatures. In 6 of the long run experiments (group A) the initial potential fell more than 50 per cent upon stimulation, and 5 of these cases were at approximately 15°C. The loss of K was the same in these 6 experiments (average 11–14 mgm. per cent) as in the other 4 experiments at about 25°C. In the short run trials (group B), only 3 cases were seen with a potential drop below 70 per cent of the original height, and all these were in the 15°C. range.

It might be argued that the decline of potential in active nerves was a sign of fatigue, or injury to some of the fibers. We excluded the possibility of injury, at least, by stimulating 5 different sets of nerves from 1–2.5 hr. each, and then allowing them to rest. The potentials, which had been reduced by an average of 50 per cent during stimulation, showed 90–100 per cent recovery after 30–60 min. of rest. Any injury which occurred therefore was reversible and not permanent.

Conclusions. These experiments provide definite evidence that even myelinated nerves *in vitro* do lose K with prolonged stimulation. Whether activity *in vivo* is ever sufficiently intense to permit this to occur in measurable amounts is a matter of conjecture, although Fenn failed to find evidence of it. Presumably there is a tendency to lose K during activity but normally the return must be practically completed during the refractory period.

When the circulation is intact, the conditions of diffusion might be more favorable for an exchange of K. Yet Rosenblueth and Luco (1939) stimulated cat nerves with an intact blood supply for as long as 5 hours without a fall in action potential, and hence presumably without a loss of K. This might indicate that diffusion of K is more rapid *in vitro* than *in vivo*.

SUMMARY

1. Frog nerves immersed in Ringer's solution lose 11 per cent of their K (10–20 mg. per cent per hr.) during stimulation with 60 shocks per sec. over a period of 60–210 min. There is no significant change during stimulation for periods of 30 min. or less.

2. Stimulation causes no shift of water, judging from the constancy of the dry weight fractions of the nerves.

3. Soaking 2-5 hr. without stimulation causes a leak of K of approximately 8 mg. per cent per hour. No further movement of K occurs when soaking is continued to 25-100 hr.

4. Soaking 25-100 hr. increases the volume of nerve water by 9.5 per cent, thereby decreasing the dry weight fraction by 6.6 per cent, and the concentration of K in nerve water by 7 per cent.

We wish to thank Prof. W. O. Fenn for his suggestion of this problem, and his patient assistance. Dr. A. C. Young was kind enough to build our vacuum tube amplifier and to aid us with the problems of recording and stimulating.

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